CLICK CHEMISTRY FOR COLLOIDAL ASSEMBLY

BY

ASFAND WAQAR

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Materials Science and Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

Adviser:

Professor Paul V. Braun

Abstract

Click chemistry has gained significant importance over last decade as an efficient tool for doing synthetic chemistry. These set of highly efficient, high yielding and extremely resilient reactions use an approach similar to the one employed by nature, with very few by products and almost no side reactions, they work perfectly fine in a multitude of reaction conditions. This new synthetic arsenal of reactions has been successfully used in the diverse fields of drug discovery, pharmacological synthesis, polymer synthesis, surface science and materials science for achieving a myriad of goals. In this study we have used one of the best reactions from the set of reactions termed click chemistry, called Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) to develop an approach for directed colloidal assembly. Colloidal crystals are used here as a model system for studying directed self assembly of colloidal particles. This study develops a modular approach towards colloidal assembly in which click chemistry is employed as an assisting agent in an attempt to devise a layer by layer route for creating colloidal crystals with crystal structures that are not readily synthesizable using conventional approaches like simple vertical deposition or spin coating alone.

This study mainly focuses on providing the proof of concept for successful incorporation of click chemistry in the process of colloidal assembly, and formation of click reaction assisted monolayer, which can be further used to create colloidal crystals in a layer by layer manner For achieving this goal several surface functionalizations were studied, for the glass and silica substrates, as well as for colloidal particles of polystyrene (PS) and silica. A couple of surface functionalization approaches are

utilized for achieving alkyne surface functionalization of glass substrates, and colloidal PS-PEG-azide and silica-azide particles were made using commercially available PS-COOH particles, and lab made silica particles. Assembly of these azide functionalized particles was then studied using two different colloidal assembly systems. The first system used sedimentation to study the viability of surface click chemistry for colloidal systems, and it was found that click chemistry can be efficiently used as a chemical tool to anchor PS-PEG-azide particles on the glass-alkyne surfaces. Patterned colloidal arrays are also fabricated, and a number of control experiments are done to provide sufficient proof that click chemistry can be used to anchor colloidal particles to the substrate. Once it was established that functionalized colloids and appropriately functionalized surfaces can be used to anchor these colloids on the surface the second system was studied. The second system was based on a technique called vertical deposition which can be used to create opals and colloidal crystals of various types of microspheres.

Vertical deposition of Silica-azide particles as well as PS-PEG-azide was done on alkyne functionalized glass substrates. Multilayered colloidal crystals were successfully created, and then the upper layers were washed away to obtain a colloidal monolayer. The idea under study here was to find out if click chemistry can be used to create a colloidal crystal using a layer by layer approach. This layer by layer approach, described in the study will be able to utilize particles of two different alternating functionalities. A monolayer of each type of particle will be formed step by step on a functionalized substrate, where in each step it is locked in the structure while still providing chemical functionality for the next layer above it to be anchored on it. The first

monolayer of Silica-azide was successfully fabricated in this study, thought its quality can be greatly improved. Several experiments are discussed here which attempt to improve the quality of the first monolayer and important recommendations are given.

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Chapter 1

Introduction

1.1 Directed Self Assembly

Self-assembly is a process in which ordered aggregates are formed spontaneously from discreet components either separate or linked due to direct specific interactions or indirectly through their environment. [1,2] Abundantly utilized by nature as an essential tool for creation, self assembly has been extensively studied all across the spectrum of scientific fields for last two decades resulting in great technological achievements. Biologists and chemists have known molecular self-assembly for a greater portion of last century, as nature's way of forming ordered and well structured functional aggregates. Where molecular synthesis depends on formation of covalent bonds to create these molecules, molecular self-assembly usually uses non-covalent interactions to arrange these molecules into ordered ensembles. Some of these self assembled molecular ensembles form an integral part of life at cellular level. Self-assembly is not just limited to molecular level but has also been successfully employed synthetically at various length scales. Especially with the advent of nanotechnology it has become a well established route to create functional materials and architectures which are not available or at best difficult to achieve using conventional approaches of synthesis.

Self assembly is driven by the principles of thermodynamics and is often associated with thermodynamic equilibrium; therefore the organized structures are broadly characterized as being in a state of minimum free energy.[3] This thermodynamic

dependency in a way limits the available structures and organized architectures achieved via self assembly to only those at equilibrium. Directed self-assembly (DSA) is an approach that employs same basic principles of self-assembly, however tweaked in an attempt to achieve novel non-equilibrium structures and architectures, free from the constraints faced by self-assembly these structures could then occupy a local state of equilibrium.

Several approaches have been utilized over the years to help attain directed self-assembly. Numerous approaches to DSA involve manipulating molecular interactions at the component level like van der Waals,[4] electrostatic,[5,4, 6] magnetic,[7, 4] and even utilizing capillary forces.[8] Other versatile techniques involve modifying surfaces to create templates, which can be either in form of additional building blocks that assist in assembly like carbon nanotubes [9] or selective chemical modifications of the surfaces by particular molecular bridges like DNAs that assist in assembly.[10-13]

Several assembled structures and architectures can been created using these techniques and approaches for directed self-assembly. Various architectures have been assembled using these approaches to self assembly, like van der Waals as driving force was utilized to give highly ordered two-dimensional hexagonal array formed by gold nanoparticles (Figure 1).[14] Electrostatics has been used to attain self assembled structures created by polystyrene (PS), where two different charged particles of different sizes were assembled (Figure 2).[5] From the array of techniques that fall under the templating or surface modification categories, DNA hybridization has been successfully used as a directing agent coupled with vertical deposition to obtain directed self-assembled structures made by PS colloidal particles of 100nm (Figure 3). [11]

1.2 Colloidal Assembly

Self-assembly of colloids and colloidal particles proves to be a very interesting case in the study of self-assembly, as these particles can be assembled using various techniques, and the resulting assembled structures have found great technological applications. Colloidal assembly used to make self assembled structures like colloidal crystals is often used in creating technologically important materials like photonic crystals.[15] Techniques employed to achieve these self-assembled colloidal structures like direct writing have become a strong contender as an alternative to microfabrication techniques like conventional lithography etc.[16, 17] One such technique frequently used to obtain colloidal crystals is called vertical deposition or control drying, and has gained importance in fabrication of photonic crystals.

Colloidal crystallization via vertical deposition (control drying) a Langmuir-Blodget like process provides an easy and robust route for making colloidal crystals specially for photonic applications, however structures that can be made are limited [18]. Monodisperse colloidal systems almost always preferably assemble into a close packed cubic crystal specifically FCC [19] and non-FCC structures have been limited to a few layers [20-23]. FCC cubic crystals obtained commonly via vertical deposition are relatively easier to make and have significant photonic properties but suffer from a relatively narrower band gap and also require a higher refractive index contrast for optimum performance.

The diamond structure is theoretically predicted to have a complete band gap and great photonic properties at a relatively low refractive index contrast. For about a decade, creating a non-closed packed; ideally diamond like structure has served as the

Holy Grail for photonic crystal community. Apart from numerous holographic or lithographic techniques, several approaches beyond conventional self assembly have been explored to assist in obtaining diamond like structure via assisted or directed self-assembly. Patchy particles having selectively functionalized regions on the surface were created to utilize varying surface geometry to help in directing the self-assembly (Figure 4).[24] Non-FCC structures have also been created by using two set of particles with each having different surface charges in an ionic solution (Figure 5), but to make useful materials out of them they must be dried, and as these particles do not touch they won't be stable out of the solution. [25] Several other approaches to directed self-assembly have also been studied, where one famous approach is to functionalize the surface of colloidal particles with suitable molecules that would further react and assist in assembly.

1.3 DNA assisted Colloidal Crystallization

The approach of functionalizing the surface with molecules has been particularly successful in case of using deoxyribonucleic acid (DNA).[11, 26, 27] DNAs ability to specifically hybridize with only complimentary DNA strands provided the ability to selectively use them as anchors on the substrate which would attract particles functionalized with alternative but complementary strands. DNA hybridization approach has been used to pattern Silica particles on glass surfaces (Figure 6), [28] and also used to assemble PS particles DNA functionalized with complementary strands on the surface of the glass substrate[11]. Significant amount of research has been done to study DNAs as directing agents or as scaffolds to help in directed self-assembly, [26, 27, 29-31] and

our group has previously successfully shown that DNAs can be used to assist in colloidal assembly specifically directed towards creating colloidal crystals. DNA ligation (hybridization of complementary strands on particles and substrate's surface) coupled with vertical deposition was used to create a monolayer, followed by formation of a second layer following the same procedure for assembling the first monolayer (Figures 7 and 8). This protocol was fairly successful however use of DNA imposed some processing limitations. The process must take place under DNA friendly conditions, like low ionic strength aqueous solutions, and the cost associated with use of DNAs was also an important factor to be considered. Therefore by the end of this study it was proposed that other chemistries must be studied for the purpose of directing self-assembly of colloids, and especially those that can be incorporated in vertical deposition. Advances in the field of click chemistry since its inception in 2001 compelled us to look at click chemistry as a viable replacement of DNA hybridizations and ligation.

1.4 Click Chemistry

In 2001 Kolb, Finn and Sharpless published their seminal paper, detailing few good reactions that provided diverse chemical functionality, and coined the term "click chemistry" to denote these extremely useful reaction schemes.[32] Since then, "click chemistry" has grown from being a mere list of reaction schemes to a mini-field in its own right, with over two thousand citations just for that very first 2001 paper, it has found diverse applications all across the synthesis spectrum, from drug synthesis and polymer chemistry, to all the way in materials and surface science.

Taking guidance from nature's way of creating biomolecules and keeping in sight a 'process chemistry' approach, Sharpless et al. described a set of stringent criteria for a reaction to qualify to be called "click chemistry". This reaction must be stereospecific (not necessarily enantioselective), wide in scope, modular, must give very high yields, and should produce only inoffensive by-products if any at all, where as these by-products should be easily removable by non-chromatographic methods like crystallization and or distillation. In addition the reaction process characteristics must include simple reaction conditions, for example the reaction should be insensitive to oxygen and water, must utilize readily available starting materials and reagents, must use no solvent, or a benign solvent like water, or an easily removable solvent, with simple product isolation.[32, 33] The general strategy for click chemistry was defined as an approach which is essentially modular, and employs small molecules that can be quickly stitched together to form complex functional compounds, such as in nature proteins and complex carbohydrates are formed by joining smaller modular units. Sharpless et al. presented click chemistry as a set of reactions that can be envisioned to be "spring-loaded" for single trajectory, as these reactions are driven by a high thermodynamic driving force, usually greater than 20 kcal/mol, and so complete rapidly and selectively to produce a single product.

Three classes of reactions were singled out as ideal candidates for "click chemistry", that include, 1) nucleophilic opening of spring-loaded electrophiles, like epoxides, and aziridines, 2) mild condensation reactions of carbonyl compounds for example hydrazones and oximes from aldehydes, and 3) cycloaddition reactions. From the cycloaddition class of reactions, a Huisgen 1,3-dipolar cycloaddition was found to be

the most useful type of reaction among all the click reactions. This specific reaction of organic azides with terminal alkynes has been called the cream of the crop of click chemistry by several papers. This 1,3-dipolar cycloaddition reaction has been known for almost past 100 years and was extensively studied in early 60s by Prof. Rolf Huisgen.[34] In 2002 a more reliable Cu(I)-catalyzed stepwise variant was independently discovered by Valery V. Fokin and K. Barry Sharpless, and Morten Meldal.[35, 36] This catalytic process has also been found to have an unprecedented level of regioselectivity.

1.4.1 Cu Catalyzed Azide Alkyne Cycloaddition

This catalyzed variant of Huisgen 1,3-dipolar cycloaddition is a sped up version of the same reaction between a terminal alkyne (an acetylene) and an azide (a 1,3-dipole of nitrogens) to form a 1,2,3-triazole as depicted by Richard A Evans, in figure 9.[37] The original Huisgen reaction before the use of copper(I) catalysis required higher temperatures for longer periods of time, and the process was non regiospecific with two possible isomers as products. The Cu catalysis not just helped to increase the speed of this reaction but also made it exclusively regiospecific, with 1,4-triazole isomer as the only product. As mentioned earlier Meldal et al published their findings in 2002, [36] that elucidate the use of Cu(I) in the cycloaddition reaction for triazole synthesis on a solid phase, but they used an organic solvent for this reaction. Shortly after it was followed by a paper from Fokin and Sharpless et al. showing that this reaction could also be water based using sodium ascorbate along with copper sulfate, which provides Cu(I) for the reaction, as these reaction schemes are shown in figure 10.[35]

Cu(I)-catalyzed azide alkyne cycloaddition has been extensively studied since then for a number of applications. A series of three papers from the same pioneering group of Sharpless et al. go by the title of “click chemistry in materials synthesis”, and the first paper in this series covers the use of click chemistry in synthesis of adhesive polymers. [38] The second one reports the synthesis of acid-swellable crosslinked polymers made by using Cu(I) catalyzed azide alkyne cycloaddition [39], and the third one covers the synthesis of metal adhesing polymers[40]. A number of great review articles and papers, also cover these diverse applications of click chemistry, like Binder and Sachsenhofer discuss the use of click chemistry in polymer and materials science.[41],[42] Right in its very first year click chemistry found great applications in fields like pharmaceutical sciences,[43,44] drug discovery and synthesis,[44] and polymer bioconjugation.[45] Cu(I) catalyzed azide alkyne cycloaddition reaction has also been successfully used in creating self assembled monolayers on various surfaces [46,47,48] and for creating functional dendrimers.[49,50,51,52]

These various applications of click chemistry and specially the use of Cu(I) catalyzed azide alkyne cycloaddition in achieving interesting surface modifications and functionalizations[50,53,47,54,48,55] served as a major motivating factor for us to explore its possible use in assisting self assembly of colloidal crystals.

1.5 Click Chemistry for Directed Colloidal Assembly

The motivation behind using click chemistry as an assisting tool for achieving directed colloidal assembly came from our group’s previous experience with using DNAs as an assisting agent. A set of characteristics were identified for choosing the

next system to be implemented, where orthogonality of these functional moieties was determined to be one of the major criteria, so that these functional linker molecules (assisting agents with terminal azide or alkyne) can be covalently attached to the surfaces and we could still utilize the terminal azide or alkyne functionalities available. The second important factor established was the catalytic nature, where the assembly assisting reaction step should specifically and selectively take place under one set of conditions, and should not take place otherwise, just like the purpose served by using complementary strands of DNA. The resulting linkage and bond should be stable under various conditions, like the bond created should not be hydrolyzable, so that further processing if required can be done in aqueous conditions. Using DNAs for colloidal assembly also has a huge monetary hindrance, if it were to be commercialized, as for such experiments specifically designed DNA strands were employed. So the new system should be cheaper and ligands or linker molecules to be used should be cheaply available or easy to make in our labs unlike DNAs.

Click chemistry was chosen as a perfect candidate based on these guidelines. Specifically Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) was considered to be the best available option. A scheme (figure 11) was created in which these functionalities would be imparted on the surface of colloids and on the supporting substrate surface, and then colloidal assembly was to be studied. As our lab group has vast experience with using vertical deposition (VD) for making colloidal crystals, it was chosen as a suitable option to study the viability of CuAAC as an assisting agent.

The experimental scheme developed (schematic shown in figure 11), involved functionalization of particles with one of the either functionalities involved in CuAAC,

that is either azide or alkyne, and then functionalization of the surface with the other alternative. Next step involves assembly of these particles on the surface, followed by a catalyst driven step where the actual click reaction takes place. In the case where vertical deposition is used for colloidal assembly, this catalytic step is where the first monolayer formed is anchored to the surface via a strong 1,2,3-triazole linkage, formed as a result of CuAAC. As vertical deposition results in a multi-layered colloidal crystal, therefore, after the first layer is anchored its necessary to remove all the layers above that are not anchored and have neither undergone click reaction. For this step several approaches were studied, which included washing in a Tween-20 solution, shaking it by using belly button shaker in the same Tween-20 solution for several time intervals all the way up to an overnight washing step. The basic advantage of having this modular approach to assembly is that, after forming the first layer, this whole process can be repeated for forming second layer, and the layers above. The underlying concept behind this approach is that, once its established that click chemistry is a good tool for directed colloidal assembly, this modular scheme can be used to form structures or colloidal crystals which are not readily available by using conventional methods of colloidal assembly like simple vertical deposition. This can be done, by utilizing colloidal particles of different sizes, in different assembly (VD) steps, and even particles made of different materials can be used, where one or the other type of particle can be selectively removed later on.

1.6 Figures

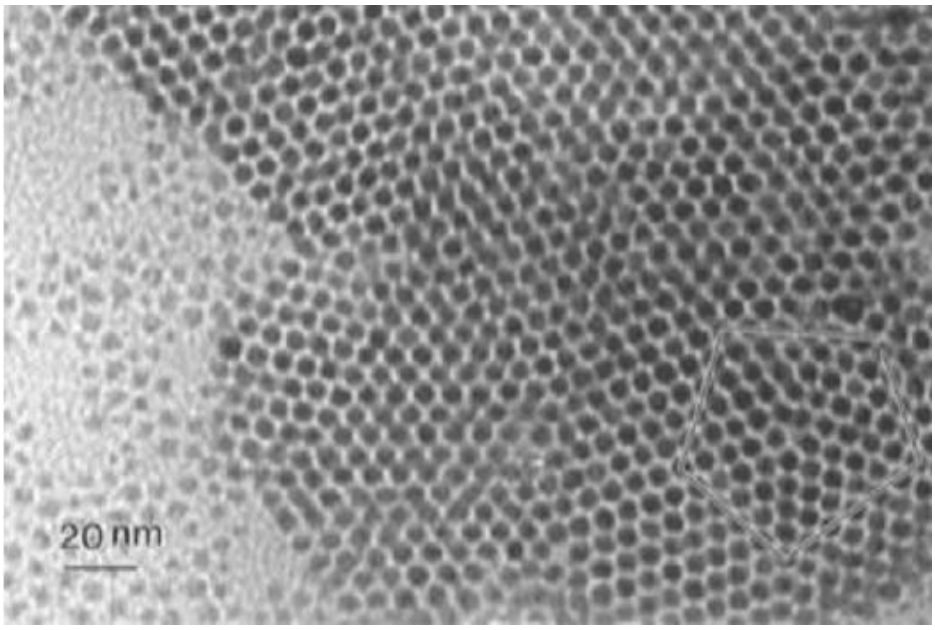

Figure 1.1 Low magnification TEM image of 2-D assembled Gold nanoparticle/nanocrystal array, adapted from Whetten et al. [14]

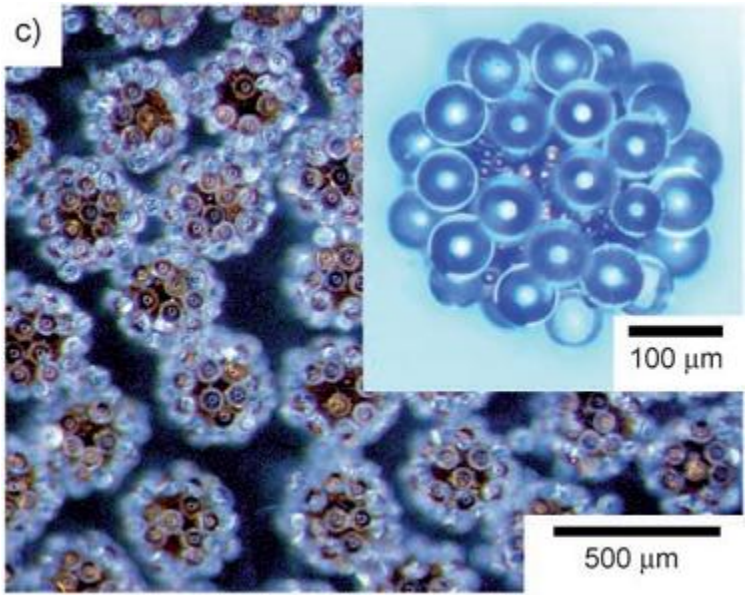

Figure 1.2 Optical micrographs of self-assembled PS spheres of diameter 200 µm and 20 µm with positive and negative charge relatively, assembled using electrostatics to direct the assembly, image adapted from McCarty et al [5].

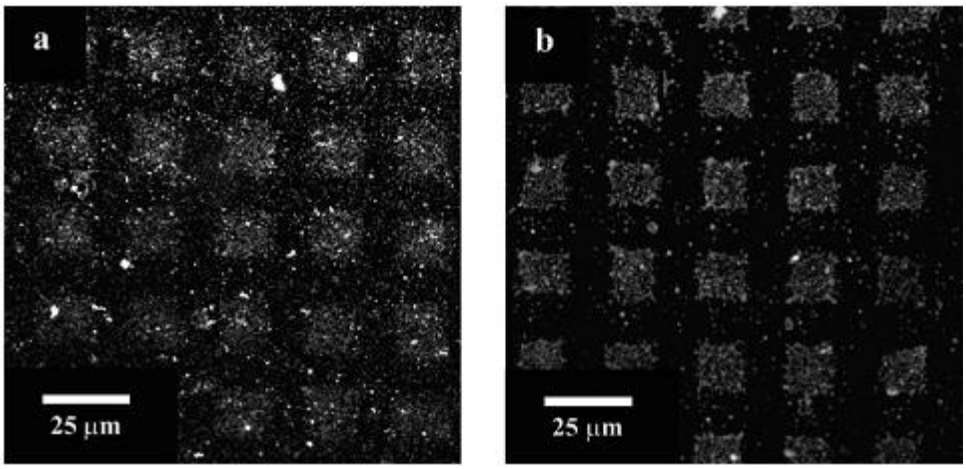

Figure 1.3 Confocal micrographs of DNA assisted self-assembled 2-D arrays of 100nm PS colloidal particles, a) imaged in buffer b) after 15 mins of drying in air, image adapted from Shyr et al [11].

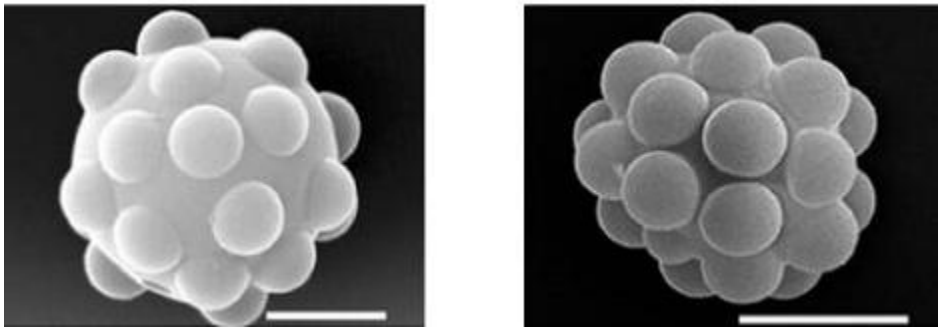

Figure 1.4 SEM micrographs of patchy PS particles, adapted by Cho et al. [24]

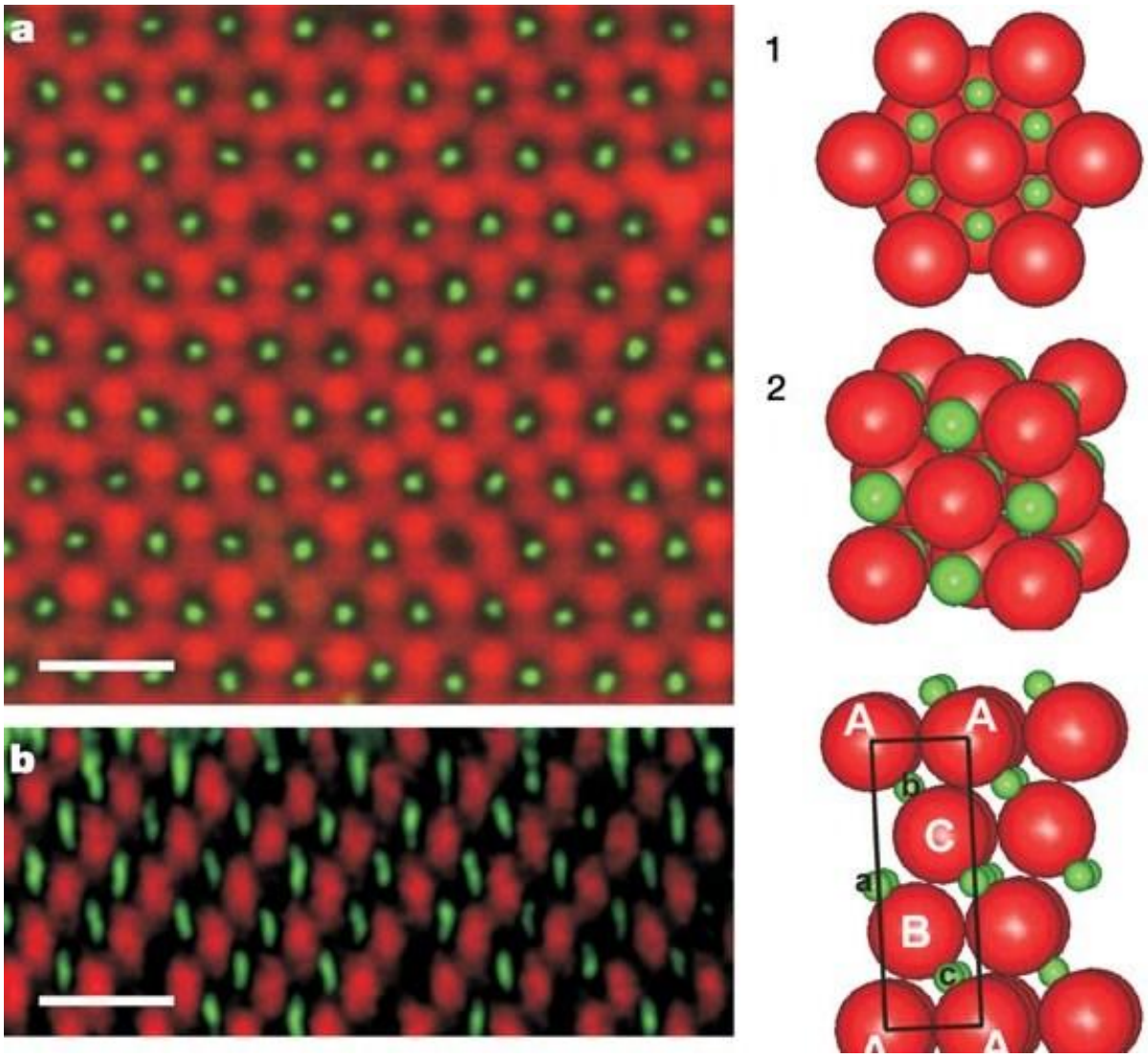

Figure 1.5 Confocal micrographs of non-closed packed NaCl type structures, where red particles are charged (radius 1.16 µm) and green are uncharged (0.36 µm) PMMA-particles in CHB-decalin. Image adapted from Leunissen et al [25].

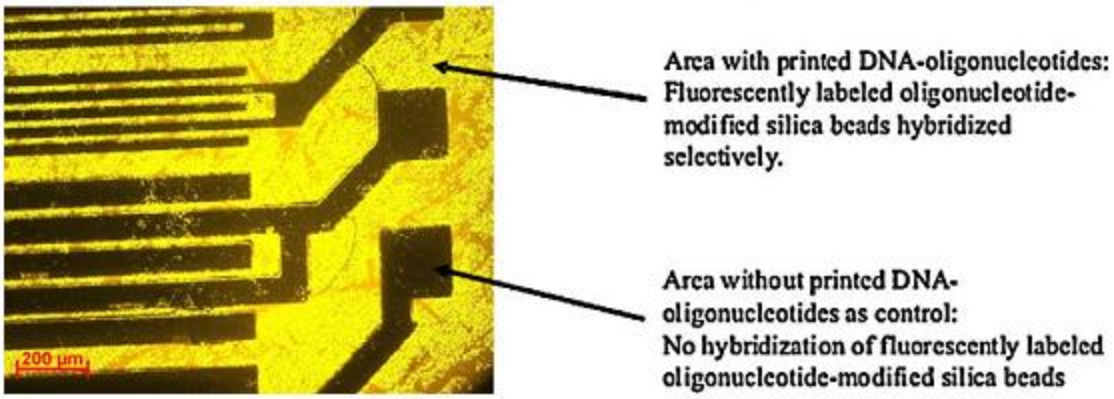

Figure 1.6 20x Optical Fluorescence micrograph showing fluorescent labeled oligonucleotide-modified silica beads hybridized selectively to the complementary oligonucleotides attached on the epoxy-glass surface. Image adapted from M. Alberti et al. [12]

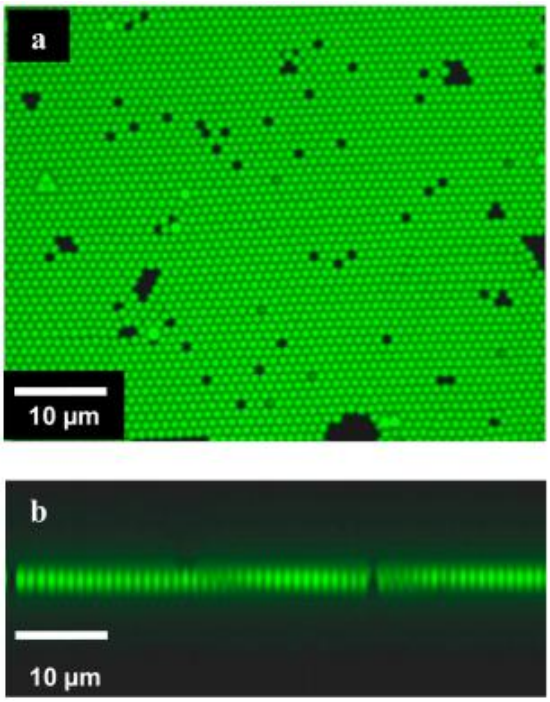

Figure 1.7 Confocal micrograph of colloidal monolayer formed by vertical deposition of silica particles assisted by DNA ligation on glass surface, a) top view, b) side view. Image adapted from PhD thesis of Margaret H. S. Shyr, 2008.

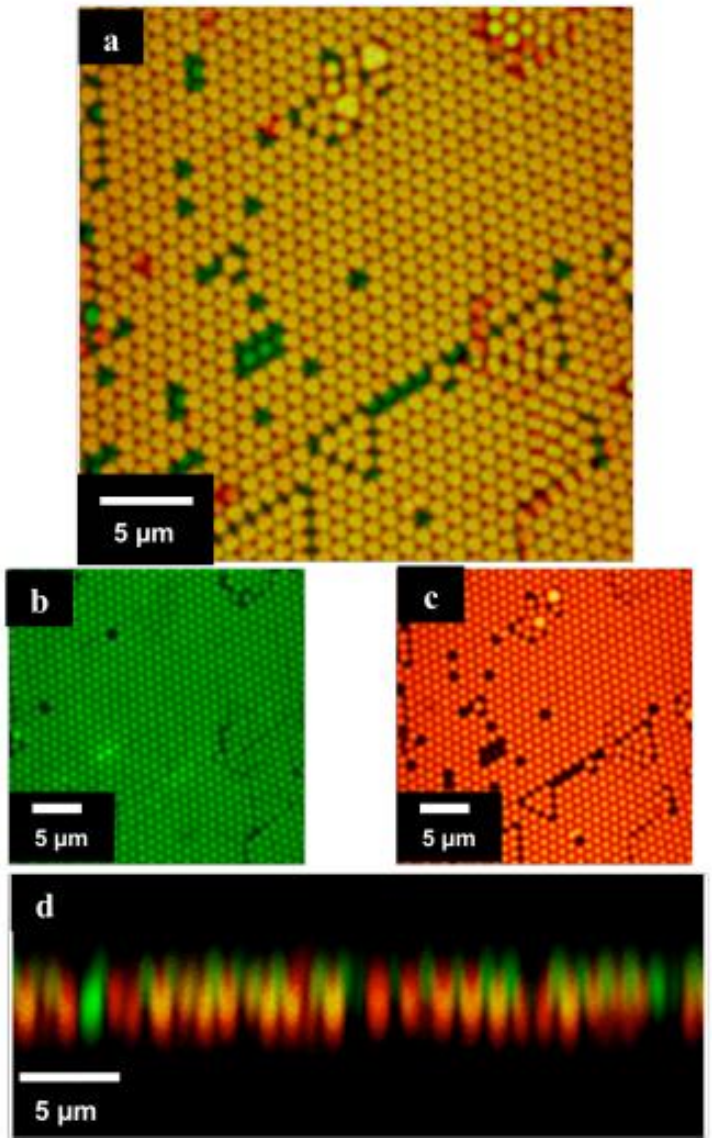

Figure 1.8 Confocal micrographs of two layer colloidal crystals formed by DNA ligation assisted vertical deposition, a) overlay of layers 1 and 2, b) layer-1 of 1µm green-yellow PS colloids, c) layer-2 of 1 µm orange PS colloids functionalized with complimentaty DNA, d) side view of two layers. Image adapted from thesis of M. Shyr 2008.

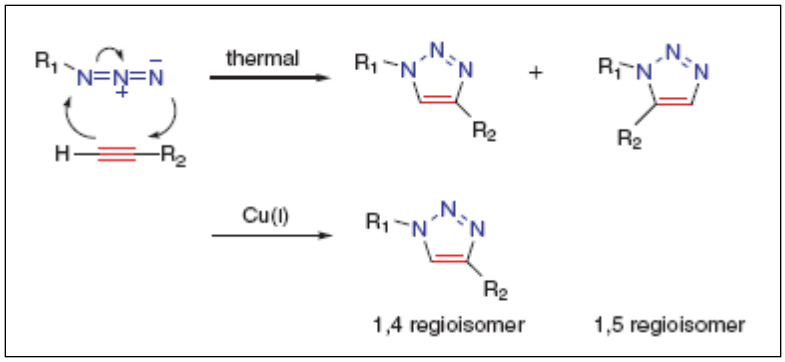

Figure 1.9 The 1,3-cycloaddition of an azide and terminal alkyne to produce a 1,2,3-triazole. As shown by Evans in this schematic that thermal cycloaddition is non-regiospecific, where as the Cu(I) catalyzed reaction is 1,4-regioselective [35, 36]. Image Adapted from Richard A. Evans Ref. [37]

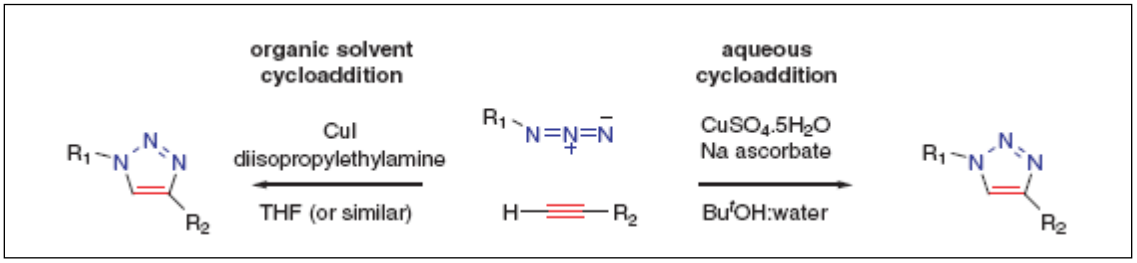

Figure 1.10 Two variants of Cu(I) catalyzed azide alkyne cycloaddition, as published by Meldal et al showing the organic solvent route on the left hand side[36] and the water based reaction published by Sharpless et al. on the right[35]. Figure adapted from Evans[37].

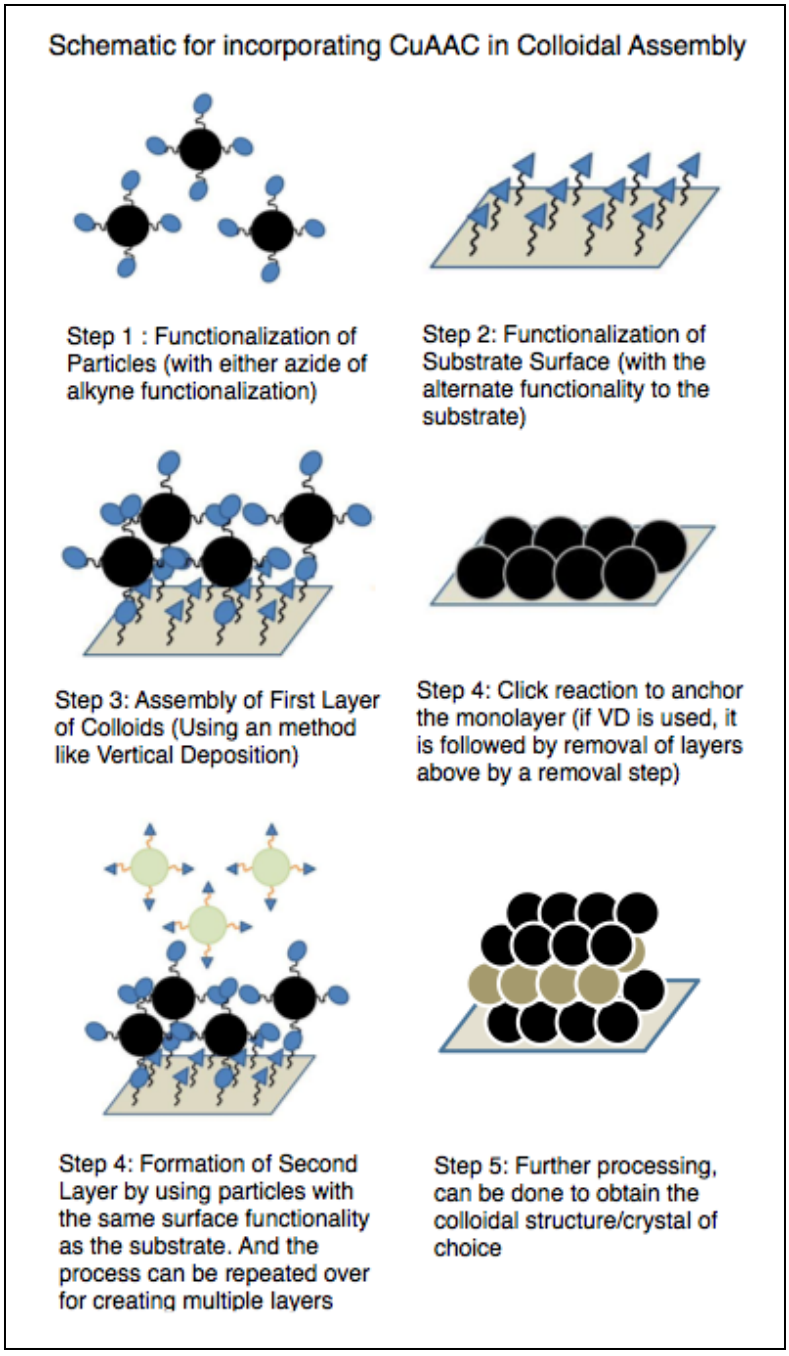

Figure 1.11 Schematic devised for incorporating click chemistry in the process of colloidal assembly. In step 3, if vertical deposition is used to obtain the colloidal crystal, as that results in formation of multiple layers, therefore in step 4, followed by click reaction a washing step is necessary for removal of layers above the first monolayer. This process can be repeated in cycles up to step 4 for forming multiple layers.

1.7 References

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Chapter 2

Click Chemistry for Colloidal Assembly via Sedimentation

2.1. Introduction

Cu(I)-catalyzed Azide-Alkyne Cycloaddition in theory provides all the suitable characteristics required to incorporate it in a system that can be used to achieve directed self assembly of colloidal particles. The task at hand then is to establish a route to functionalize particles, the substrate and then study if this system is workable or not. The main question to be answered here is to figure out if the click reaction between azide and alkyne on these surfaces is actually going to anchor these particles to the surface or not, and to observe the effects caused by incorporating click chemistry in the process of colloidal assembly. This chapter mainly covers experiments that provide the proof of concept, and help establish experimentally that click chemistry is a potential tool for assisting in colloidal assembly.

There are various studies and papers published about click chemistry on surfaces, especially related to utilizing Cu(I)-catalyzed Azide-Alyne Cycloaddition as an efficient tool for doing surface chemistry.[37, 54] These papers cover a wide range of surfaces and a variety of functionalization routes, and strategies. One particular recipe entails functionalization of silicon surfaces utilizing 1,8-nonadyine as the molecule of choice for functionalization, where this diyne molecule's one terminal alkyne bonds with the surface, and the other provides free terminal alkyne for further use in a click reaction.[56, 56, 56, 56, 57, 57] Another approach includes

functionalization of silica substrates with 11-bromo-undecylsiloxane forming a SAM, which is further treated with sodium azide to substitute the terminal bromides with azides, resulting in a azide terminated SAM.[58] Other reports include functionalization of silicon substrates using a two step chlorination/alkylation method.[59] Silica nanoparticles have also been functionalized with alkynes, involving an intermediate functionalization with an amine terminated self assembled monolayer, which was further reacted with propargylchloroformate to yield alkyne funtionalized silica nanoparticles.[60] Several other studies involve synthesis of an alkyne terminated silane molecule which can be further used to form a SAM on the surface of silica particles, and use of an intermediate chlorine functionalized SAM to react with sodium azide to yield azide functionalized silica particles.[61] Apart from these silica and silicon surfaces several other surfaces have also been studied and functionalized appropriately for click chemistry, including synthesis of poly(propargyl-acrylate) polymer colloidal particles having alkyne surface functionality.[62] Another interesting approach utilizes a step involving vapor deposition of 11-bromo-undecyltrichlorosilane (BUTS), on silica surfaces, and in microfluidic glass channels, followed by a treatment with sodium azide to modify bromo-terminated surface into azide terminated via SN2 nucleophilic substitution.[63]

2.2 Experimental

In these experiments glass substrates are functionalized with alkyne, and carboxylic modified polystyrene (PS) particles (Invitrogen) are reacted with bi-functionalized polyethylene-glycol (PEG) oligomers having a terminal azide and a

terminal amine functionality to yield PS-azide particles. These PS-azide particles are then allowed to sediment on the glass-alkyne substrates, followed by a catalytic (click reaction) step, in which these substrates are transferred to a copper catalyst solution. Followed by click reaction they are thoroughly washed to clean away all the unreacted particles, and the samples are finally observed under a fluorescent optical microscope. To validate the process simultaneous control experiments are also done. A process to form patterned surfaces is also utilized, in which particles selectively react with the alkyne functionalized surface, and don't reach with the control octadecyltrichlorosilane (OTS) patterned surface. All the chemicals are purchased from Sigma-Aldrich unless otherwise stated, and are used without further processing. The carboxylated-PS particles are used are purchased from Invitrogen Inc., from their set of available fluorospheres, and are washed in deionized Milli-Q (DI) water before further use as mentioned in the recipes below.

2.2.1 Alkyne Functionalization of Glass Surfaces

There are several protocols available for alkyne functionalization of silica and glass surfaces, we utilized a modified version of one of these existing recipes in literature and created another approach based on a reaction widely used in bioconjugation. The first recipe that uses propargyl chloroformate is partially adopted from Prasad et al. and modified for using on glass substrates.[60]

The first step in over all glass functionalization involves functionalizing glass surface with an aminopropyl-triethoxy-silane, followed by a second step involving a reaction

with a) propargyl-chloroformate or b) 4-pentynoic acid. It is the second step that differentiates between the two recipes.

A. Amine Functionalization of Glass Surfaces

For amine functionalization of glass cover slips one of the recipes studied by Youngblood et al.[64] was employed. Glass cover slips were cleaned using piranha etch (Sulphuric Acid and Hydrogen peroxide in 3:1) for 30 mins, followed by a step involving thorough washing of the substrates with copious amounts of DI (Milli-Q) water and then the substrates were dried under a spray of nitrogen. A 25 ml solution of 1% 3-aminopropyltriethoxysilane (APTES) in ethanol was prepared, and the dried cover slips were immersed in this solution in a Petri dish. The reaction was allowed to go on for 2 hours to form a uniform self assembled monolayer providing terminal amines for further reaction on the surface of the substrate. Cover slips were washed using copious amounts of DI (Milli-Q) water, followed by washing with copious amounts of ethanol to remove leftover unreacted silane molecules and then dried using a stream of nitrogen.

B. Alkyne Functionalization

a) Reaction with Propargyl Chloroformate

Amine functionalized cover slips were then immersed in a solution of 20ml dry acetonitrile (ACN) containing 600 µL of propargyl chloroformate and kept under inert

conditions to react overnight. Cover slips were removed next morning, washed with copious amounts of DI (Milli-Q) water, followed by copious amounts of ethanol and dried using nitrogen. The alkyne functionalized substrates were immediately used for the next step which in this case will be sedimentation. The recipe using propargyl chloroformate is schematically represented in figure 2.2 as alternative step (a).

b) Reaction with 4-Pentynoic Acid

Alternatively another recipe was also developed, which followed a common bioconjugation route involving amide formation using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). For this a 50 mM MES buffer solution (25mL) of PH 4.6 was prepared, and the amine functionalized substrates were placed in it. Followed by addition of 122.5 mg of 4-pentynoic acid (50mM), and 0.479g of EDC (100mM). Then the amine functionalized substrates were added to this, after the solution was well mixed. This recipe was originally adopted before the publication of recipe mentioned above, and a number of samples were studied using this one (as mentioned in the results), however later on this recipe was dropped in favor of the first one as the amide forming reactions involved have low yields, and the process involves several steps that increase the overall experimental time. These substrates were immediately for the next step, which in this case was sedimentation followed by click reaction. This recipe for functionalization of silica particles is schematically shown in figure 2.2 as step (b).

2.2.2 Azide Functionalization of PS particles

Carboxyl modified 0.5 µm PS particles with fluorescent cores (fluorospheres) by Invitrogen Inc. were used for these experiments and a hetero-bifunctional PEG oligomeric spacer (Figure 2.1) was used to provide with the necessary azide functionality on the surface.

To functionalize PS-COOH fluorophores N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was used as an activating agent, to activate the surface bound terminal carboxylic acids on the PS particles, to form an amide bond with the amine ends of O-(2-Aminoethyl)-O′-(2-azidoethyl) nonaethylene glycol, an oligomeric hetero-bifunctional PEG. 50 µL carboxyl functionaized 0.5 µm PS (2% solids) particles were dispersed in 1mL of DI (Millipore) water and centrifuged at a rate of 13.2k rpm for 20 minutes and supernatant was removed, in order to get rid of the original suspension it comes in. Finally they were re-dispersed in 700 µL MES buffer solution (50mM) at PH 4.6. The bifunctionalized PEG had amine at one terminal end and azide at the other, with ten repeat units of the ethylene glycol. 50µL of this amine-PEG-azide was added to the dispersion of particles in MES buffer (60 µM), followed by addition of 10 µL of 5M EDC (6.67 mM) solution prepared in MES buffer at PH 4.6, and left on vortex mixer set on slow for 30 mins. After initial 30 mins another 20 µL of 5M EDC solution in MES was added, and the solution was left to react on vortex mixer for another 30 mins. Centrifuged after a total reaction time of 1 hour, at 13.2k rpm, for 20 mins and supernatant was removed. Redispersed in DI (Millipore) water, followed by another step involving centrifuging it at 13.2k rpm, followed by redispersion in DI to remove

unreacted reactants. These particles were immediately used in the next step, which in this case was sedimentation followed by click reaction.

2.2.3 Sedimentation and Click Reaction

Alkyne functionalized glass substrate was placed in a Petri dish, and filled with 20ml of DI (Millipore) water, followed by the addition of 1ml solution of azide functionalized PS particles prepared as shown in previous step. Particles were allowed to sediment overnight, and the next day substrates were immersed in a catalyst solution of 10mM $CuSO_4.5H_2O$ and 50mM L-Ascorbic acid for click reaction. The click reaction was allowed to go on for 5 hours, and the substrate was cleaned gently with copious amounts of DI and ethanol alternatively several times after being removed from the Petri dish, to remove any unreacted, and or untethered particles on the substrate. Several other post cleaning steps were also investigated, some of which used 5M Tween-20 solution to remove the untethered particles more efficiently while shaking it on belly bottom shaker for a variety of time periods ranging from 30 minutes to overnight.

2.3 Control Experiments

A number of control experiments were employed to study the effects of click chemistry and to find out if the results observed can be explained based on anchoring of the particles to the surface specifically caused by click chemistry or not. Control experiments were designed in several ways to study the effects of functionalization of the substrate as well as the functionalization of the particles.

This was done by following the same complete recipe given above but by changing just one of the steps in it. The control experiments studying the effects of substrate alkyne functionalizations involved the use of differently functionalized or bare piranha clean substrates, where as to study the effects of azide functionalizations of the particle, the unreacted as obtained PS-COOH particles were used as a negative control, with rest of the recipe remaining the same. Everything else in the experiment was kept exactly the same, so that only the effect of these different surface functionalities can be studied. To study the surface alkyne functionalization an azide functionalized dye, 7-(Diethylamino)coumarin-3-carbonyl azide was also used on the alkyne functionalized patterned surface to react with the surface alkynes, and then observed under the microscope following similar cleaning steps used for removing the particles.

2.3.1 Blocking via Microcontact Printing and Backfilling to Form Patterned Surfaces

To obtain patterned surfaces that can be further used in achieving patterned colloidal assembly microcontact printing was used. Microcontact printing has several advantages one of which is being cheap, and another is the potential for a high throughput for producing chemically patterned surfaces. For patterning the surfaces, steps described above to functionalize glass cover slips with alkyne were modified. A step involving stamping of the substrate with Octadedyltrichlorosilane (OTS) using a PDMS stamps was added to form a pattern of OTS on the surface which would block the APTS from reacting with the surface.

For microcontact printing a 1% OTS solution was prepared in hexane, and the solution was spread uniformly on the patterned surface of the stamp by a gentle swipe of a cotton swab immersed in the OTS-hexane solution. The stamp was then allowed to dry leaving only OTS behind. This was then gently pressed against a piranha cleaned coverslip glass substrate and held in that spot for around 20 seconds, leaving behind a pattern formed by OTS. This hydrophobic layer in next steps would prevent aminopropyl-triethoxysilane APTS from reacting with the surface hence rendering a patterned region for colloidal assembly. For backfilling the substrate and to provide it with the required alkyne functionalities same steps as described above for the piranha cleaned substrates were followed.

2.4 Results and Discussion

To observe the resulting samples prepared after following the protocols mentioned above we used fluorescence light microscopy, as PS particles used had yellow fluorescent cores. A Zeiss Axiovert 200M inverted light (optical) microscope was used in transmission mode for imaging. The samples were kept wet for these observations under the microscope, and so were just pulled out of the DI (Milli-Q) water before imaging them. This was deemed necessary as once the particles dry out; their fluorescence behavior changes and often at times they burst and split open which can lead to artifacts.

a) Sedimentation

The optical micrographs of the resulting substrates after sedimentation of PS-PEG-Azide particles are shown in figures 2.3 for substrates functionalized using propargylchloroformate and in figure 2.4 for the substrates functionalized using the 4-pentynoic acid recipe. From these images it can be seen that both of the routes seem to work, and the particles are well anchored to the surface as the cleaning steps could not wash them away. Also as further control experiments show that the absence of azide functionality on the particles, and alkyne on the substrate leads to poorer anchoring of the particles and they get washed away in the subsequent washing steps. However, it is also important to note here that in comparison between both of these routes there was no significant difference between each individual route, either using propargylchloroformate or 4-pentynoic acid.

b) Control experiments

The two sets of control experiments performed next are shown in the figure 2.5, where set (a) uses amine functionalized substrates without further functionalization steps, cleaned in a similar fashion and two sets of different particles the PS-COOH as obtained, only washed using DI water to remove the solution it comes in, and the PS-PEG-Azide particles functionalized using the recipe discussed in section 2.2.2, and set (b) shows a bare substrate piranha cleaned used with the same set of two particles PS-COOH and PS-PEG-Azide.

In these control experiments the substrates were also cleaned in a 5M Tween-20 bath by shaking on a belly button shaker, after the sedimentation step, for

30 minutes, 1 hour and overnight. The optical microscopy images shown in figure 2.6 for the amine functionalized substrates, functionalized using the same APTES recipe as described above, and the PS-COOH particles sedimented on it, followed by the same click reaction step and the Tween-20 washing step mentioned in the previous section. From the microscopy images it is observable that the surface coverage has significantly gone down when compared with the alkyne substrate and PS-PEG-Azide particles shown in figure 2.10. It is also noticeable that in this case longer times for washing in Tween-20 solution don't make an observable significant difference when compared with 30 minutes (a) sample.

In the next control experiment amine functionalized substrates were used along with azide functionalized particles (PS-PEG-Azide), and from the microscopy images shown in figure 2.7 it is clear that things aren't that clear. Though the surface coverage has gone down significantly when compared with fig 2.10, but it is still more than the last one, and so there must be some reason for the PS-PEG-Azide particles to adhere more strongly with the amine surface.

In the next set of control experiments a bare piranha cleaned substrate was used, and the particles used were PS-COOH shown in figure 2.8 and PS-PEG-Azide shown in figure 2.9. The same protocol was used for all of these experiments, as described in the experimental section. From the microscopy images shown in figure 2.8 it is clear that piranha cleaned surfaces and the carboxylic particles PS-COOH don't adhere that well, and so there is almost no surface coverage. Especially in the sample shown in figure 2.8 (c) there was no particle found at all even after observing a large area. However when observing the azide particles (PS-

PEG-Azide) on even the bare piranha cleaned surface (as shown in figure 2.9) there is a significant amount of surface coverage, though still relatively less than the alkyne-azide samples shown in the figure 2.10. This forms a pattern where it is visible that these PS-PEG-Azide particles are just more adhering and sticky on their own. As in figure 2.10 click reaction is causing them to anchor to the surface and so that is a viable explanation for them to stick there. However these particles are still getting attached to different types of surfaces like amine functionalized (shown in figure 2.7) and bare piranha cleaned glass substrate (shown in figure 2.9), and so this can be attributed to the nature of the particles. There are several hypotheses that can be further investigated, like the fact that this can be a result of the PEG spacer that's there on the particles, and it will be seen in the next chapter that we do use other recipes to fuctionalize silica particles, without this PEG spacer. It is also pertinent to note here that use of this strategy where a bi-functionalized PEG molecule was utilized to achieve surface azide functionalization was inspired by our labs last similar project in which DNAs were used. The problem over there was the nature of interactions involved, as the complementary DNAs were binding together based on the van der Waals and other secondary interactions, driving the assembly, and so overcoming the Debye length of electrostatic interactions was important, in order to allow these particles to come closer enough to the surface for interaction. So the PEG spacers were incorporated for that, and therefore in the initial stages of this project the same approach was adopted. However, as it will be seen in the next chapter that click chemistry approach still works fine, even if the PEG spacer is not involved because of the nature of the bond formed to anchor the particles to the

surface is covalent, and the Debye length is already overcome by the surface functionalizations used there, which incorporate silane molecules. Another important aspect can be related to the fact that azide is a 1,3-dipolar molecule and so what role this polarity might be playing in terms of imparting some inherent stickiness to these particles when it comes to interacting with charges surfaces, via some electrostatic interaction. Piranha cleaned silicon or glass surface is considered rich in native hydroxyls and amine functionalized surface at a PH 7 where most of the secondary steps like click reaction and sedimentation take place, is also protonated and so can carry surface charges. This can be studied by varying the PH and observing the effects on the “stickiness” of the PS-PEG-azides. For this part of the project these control experiments point towards the fact that click reaction or the Cu(I)-catalyzed azide alkyne cycloaddition can be successfully used to anchor colloidal particles to the substrates. In the following section some patterned surfaces are employed to study further potential of the click chemistry in terms of forming patterned colloidal structures.

c) Micro-contact stamped patterned surfaces

In these set of experiments the protocol discussed in the experimental section to micro-contact print the substrates was used, and hence patterned surfaces were obtained with alternating regions of alkyne surface functionalization and OTS patterned regions. These patterned surfaces were further used to form patterned colloidal arrays, and were also tested with an azide coumarin dye to test for the surface coverage. The rest of the protocol for sedimentation and click

reaction followed by washing in DI water was kept the same for forming these colloidal arrays. Steps involving washing in Tween-20 solution were not carried out for these set of experiments.

The protocol used for these experiments to impart azide functionalization to the surface was the one which utilizes propargylchloroformate discussed in section 2.2.1. The resulting colloidal arrays formed can be seen in the figure 2.11 where colloidal arrays are formed on the alkyne functionalized region of 120 µm length as a result of the click reaction forming a 1,2,3-triazole link due to the Cu(I)-catalyzed azide alkyne cycloaddition. The regions functionalized by the OTS (80µm wide lines) remain uncovered. To visualize that the colloidal array was indeed formed at the alkyne functionalized regions a dye molecule was used a control experiment. The same patterned surface was reacted with 7-(Diethylamino)-coumarin-3-carbonyl azide, the dye molecule shown figure 2.13. The brighter regions are click reacted surface attached dye molecues (120 µm wide lines) and the darker regions are OTS patterned SAM (80 µm wide) formed by micro-contact printing. The poor contrast is due to the fluorescence from the back side of the glass slide, as that was not masked and ended up being completely functionalized with alkynes.

These colloidal arrays can be further studied for a number of other applications, as well as incorporated in a process aimed towards creating exotic colloidal structures not achievable by conventional methods.

2.5 Conclusions

In this chapter a series of experiments were discussed that highlight the importance of incorporating click chemistry in colloidal assembly and provide a solid proof of concept for utilizing Cu(I)-catalyzed azide alkyne Huisgen cycloaddition. A simple set of protocols were established to functionalize the particles and substrate (glass) surfaces with suitable functionalities that can be further incorporated in a colloidal assembly process. These experiments show that click chemistry is working fine for tethering and anchoring colloidal particles to the appropriately functionalized surfaces, and so can be further incorporated in a directed assembly system. The particles used here PS-PEG-Azide did show some yet to be explained behavior which was termed as their inherent “stickiness”, in terms of preferentially attaching to the surfaces, however rest of the control experiments show that the system of particles and surfaces incorporating click chemistry performs significantly better than the negative controls, and hence is viable to be used further. The next steps to be taken forward from here include incorporation of this click chemistry system in a process that leads to colloidal assembly like vertical deposition. This system was also used to create colloidal arrays, on alkyne functionalized surfaces. However, it’s important to note here that sedimentation if looked at from a colloidal assembly point of view is not the appropriate approach, there for it is important to study other approaches to colloidal assembly, hence vertical deposition is going to be the focus of next chapter.

2.6 Figures

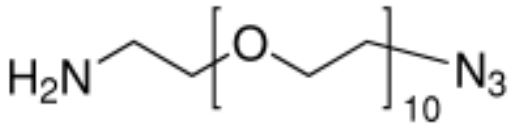

Figure 2.1 O-(2-Aminoethyl)-O′-(2-azidoethyl) nonaethylene glycol a bi-functionalized oligomeric PEG used to provide surface azide functionality to the PS-COOH particles, purchased from Sigma-Aldrich.

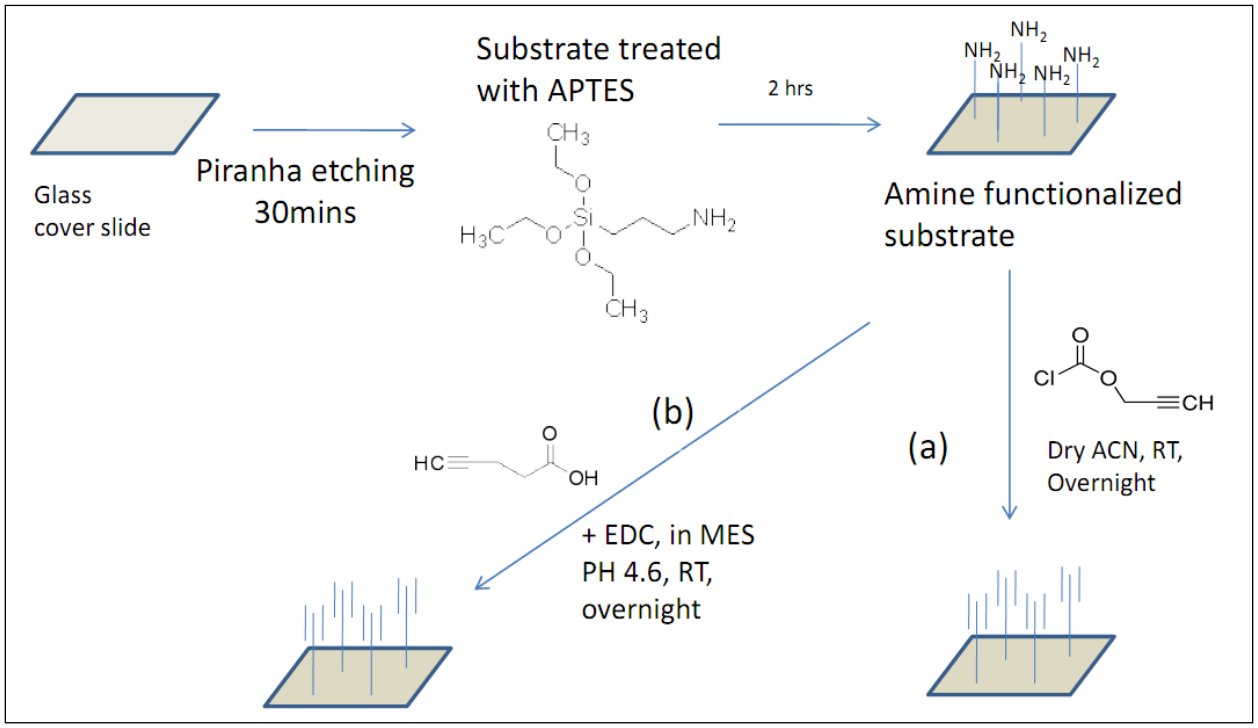

Figure 2.2 Schematic of the two recipes used for functionalization of glass substrate with terminal azide, using (a) propargyl chloroformate or (b) 4-pentynoic acid.

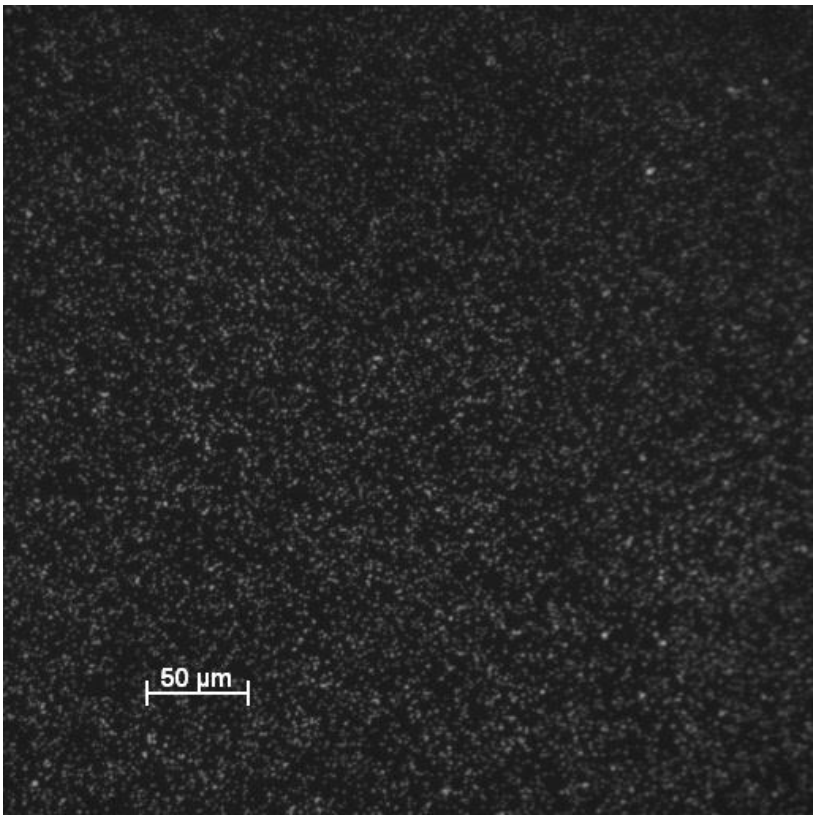

Figure 2.3 Sedimented colloidal PS-azide particles (0.5 µm) on an alkyne functionalized substrate (functionalized using propargylchloroformate recipe), imaged in fluorescent microscope after click reaction followed by thorough washing.

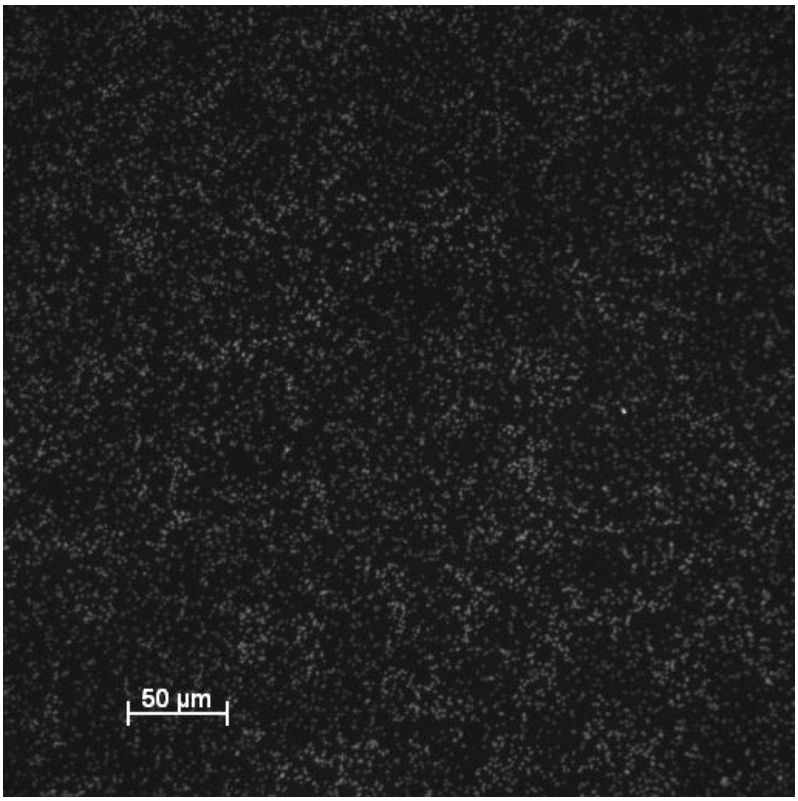

Figure 2.4 Sedimented colloidal PS-azide particles (0.5 µm) on an alkyne functionalized substrate (functionalized using 4-pentynoic acid recipe), imaged in fluorescent microscope after click reaction followed by thorough washing.

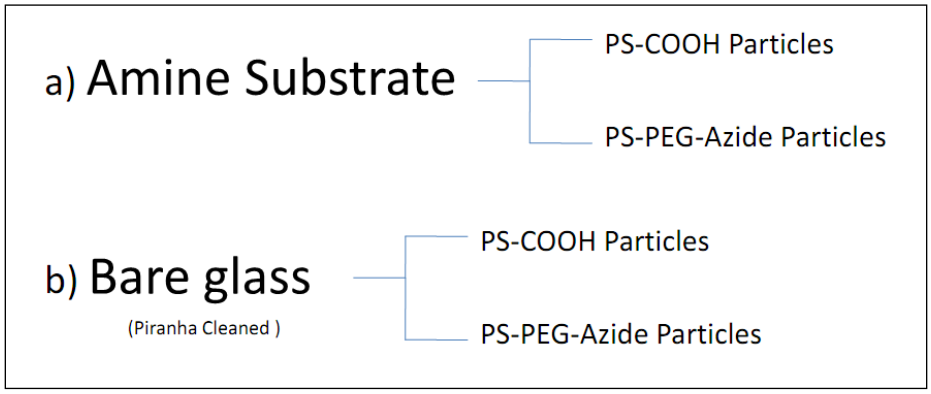

Figure 2.5 Schematic showing set of control experiments performed to verify if click chemistry was actually working.

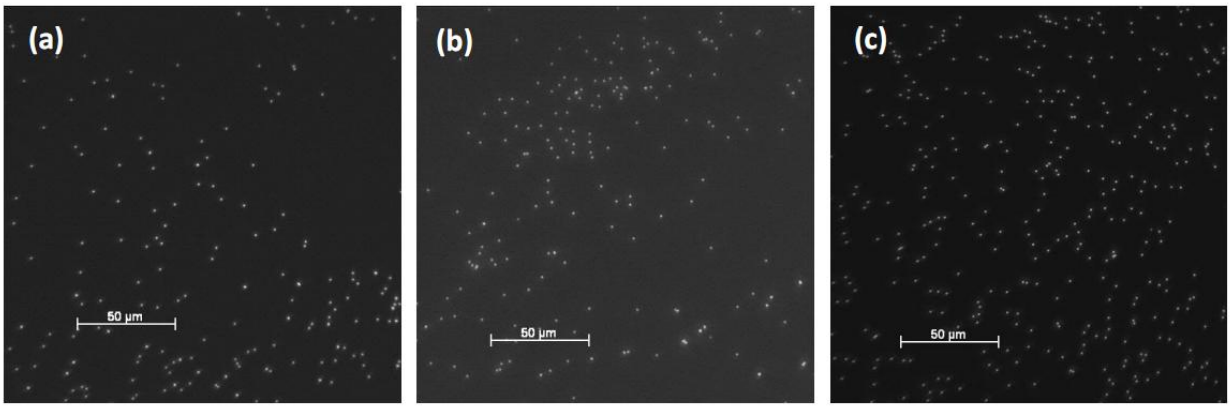

Figure 2.6 Control experiment performed with amine functionalized substrate (functionalized with APTES), and the carboxylic particles (PS-COOH), following rest of the same step for click chemistry and washed in 5M tween-20 solution for (a) 30 mins, (b) 1 hour, and (c) overnight.

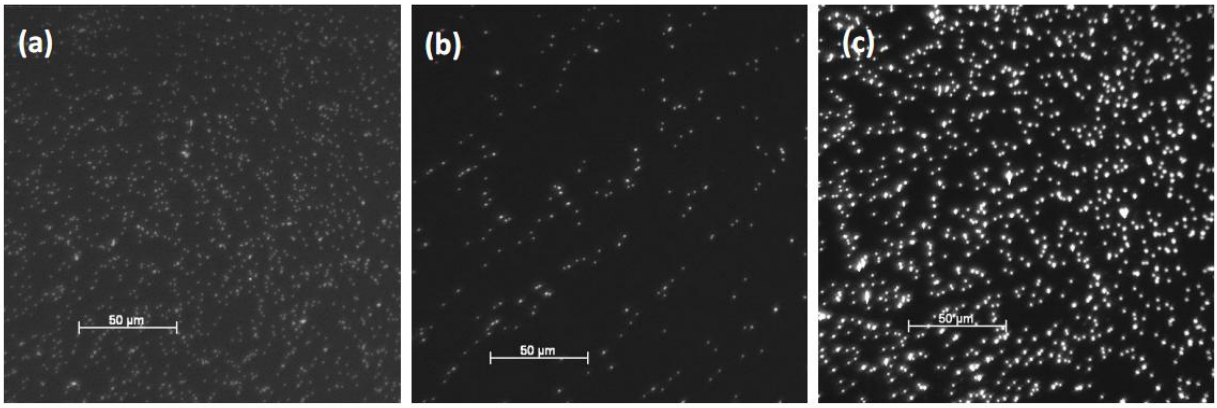

Figure 2.7 Control experiment performed with amine functionalized substrate (functionalized with APTES), and the azide functionalized PS particles (PS-PEG-Azide), followed by the same catalytic step for click chemistry and washed in 5M tween-20 solution for (a) 30 mins, (b) 1 hour, and (c) overnight.

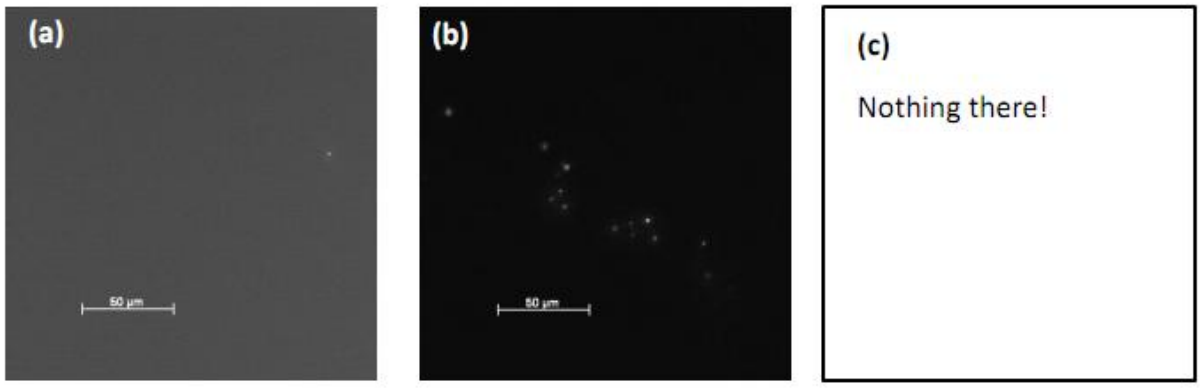

Figure 2.8 Control experiment performed with bare piranha cleaned substrate and the carboxylic PS particles (PS-COOH), followed by the same catalytic step for click chemistry, and washed in 5M tween-20 solution for (a) 30 mins, (b) 1 hour, and (c) overnight. The sample (c) showed absolutely nothing, even after scanning a larger area.

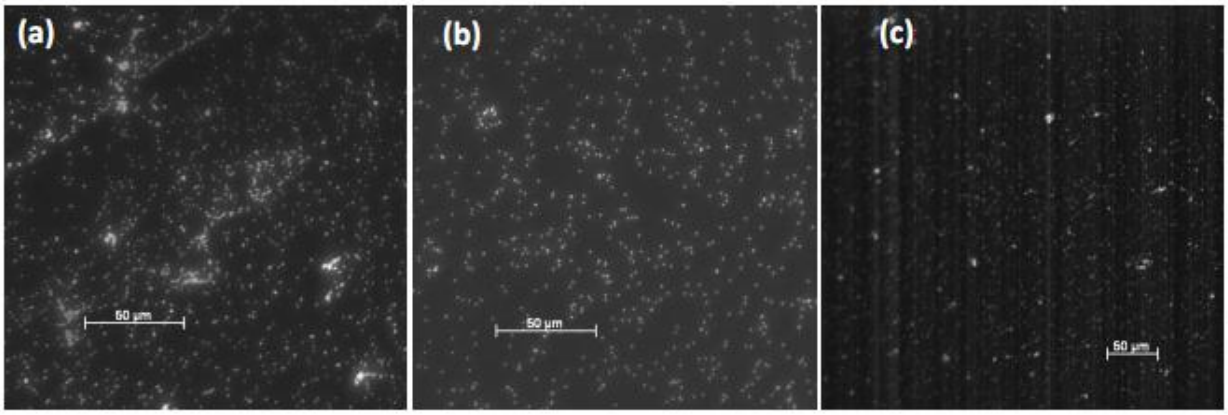

Figure 2.9 Control experiment performed with bare piranha cleaned substrate and the azide functionalized PS particles (PS-PEG-Azide), followed by the same catalytic step for click chemistry, and washed in 5M tween-20 solution for (a) 30 mins, (b) 1 hour, and (c) overnight.

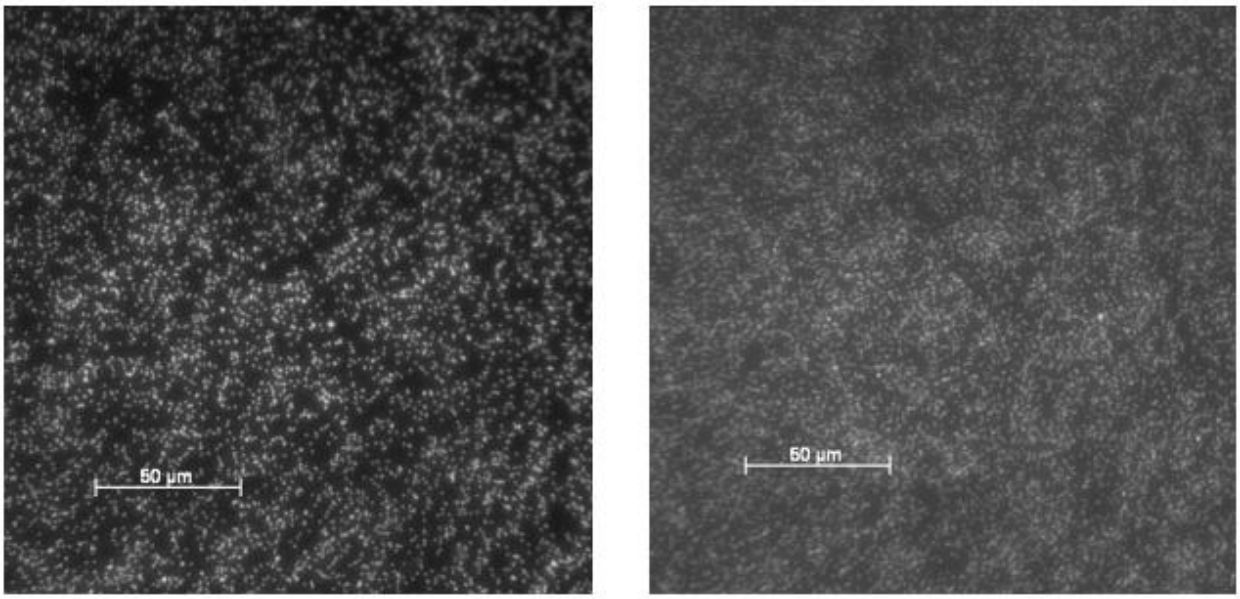

Figure 2.10 Sedimented and anchored (click reacted) PS-PEG-Azide particles on alkyne functionalized glass substrate (prepared using 4-pentynoic acid recipe), washed in 5M Tween-20 solution for 30 mins (left) and overnight (right) observed in the fluorescent microscope.

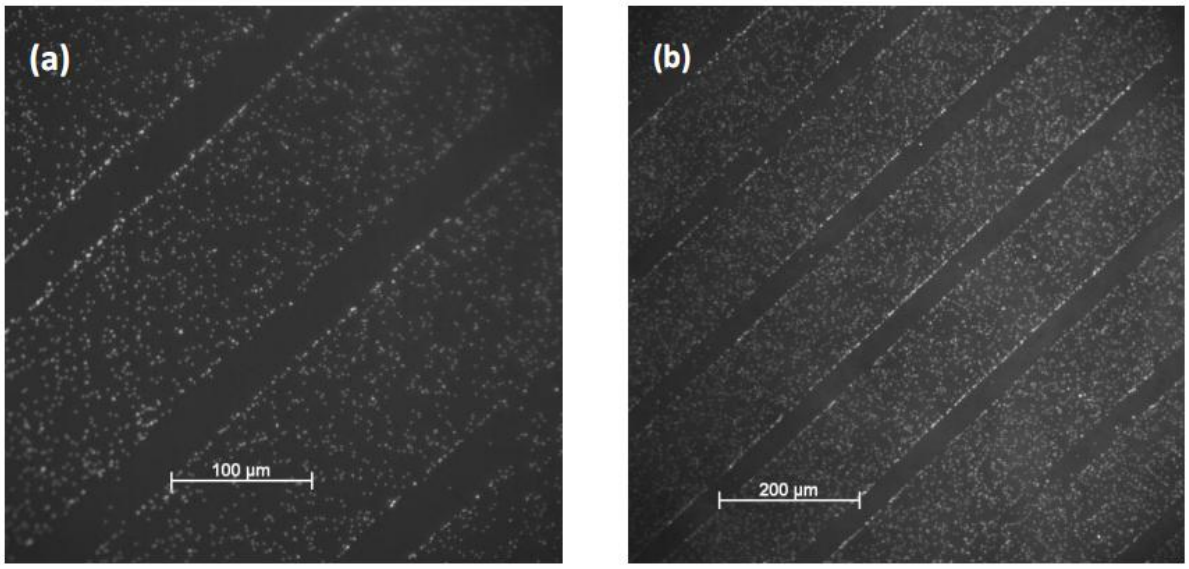

Figure 2.11Micro-contact printed OTS patterned surface, with alternating regions of azide functionalization and OTS surface coverage. The colloidal particles only anchor at the azide regions where they can form the 1,2,3-triazole link as a result of the Cu(I) catalyzed azide alkyne cycloaddition click reaction, forming colloidal arrays. The pattern used was 80 µm raised lines (OTS regions) separated by 120 µm troughs (back filled for surface aizde functionalization using propargylchloroformate protocol discussed earlier).

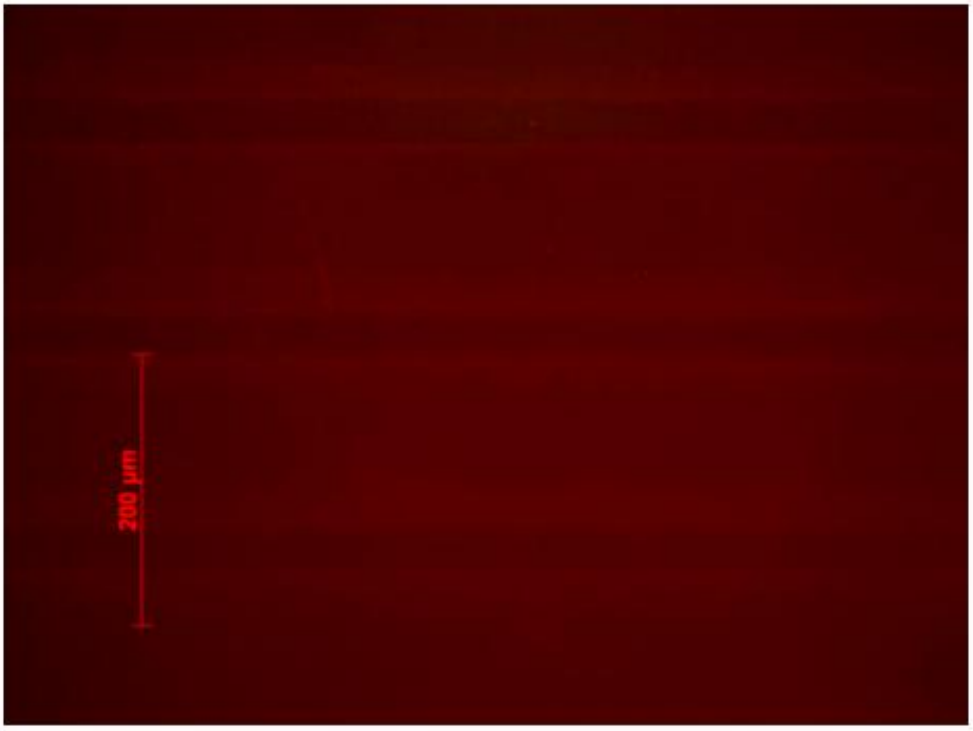

Figure 2.12 Micro-contact printed patterned surface click reacted with an azide coumarin dye. Patterned with OTS (80 µm wide lines) and alkyne (120 µm wide lines), shows that the colloidal arrays formed in figure 2.11 were indeed formed on the alkyne functionalized surface, and the dye used for this experiment was 7-(Diethylamino)coumarin-3-carbonyl azide.

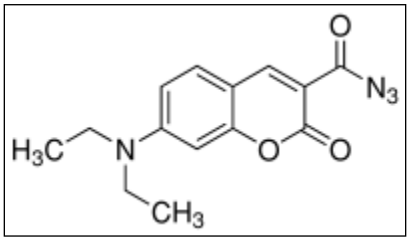

Figure 2.13 Azide dye molecule used; 7-(Diethylamino)coumarin-3-carbonyl azide.

2.7 References

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Chapter 3

Click Chemistry for Colloidal Assembly via Vertical Deposition

3.1 Introduction

In this chapter a set of experiments are studied that try to mesh up click chemistry, specifically Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) with the process of colloidal assembly via vertical deposition. As was shown in the last chapter CuAAC has great potential to help direct or assist colloidal assembly, however sedimentation itself is not such a strong self assembly tool. Vertical deposition, however has over the period of last decade and a half gained great significance when it comes to forming colloidal crystals. This fairly simple and sort of artistic process has become the bread and butter of scientists who grow opals for making various photonic structures.

3.2 Vertical Deposition

The method of vertical deposition for making colloidal crystals was first reported by Colvin et al.[65] in 1999, and since then it has been studied extensively and has resulted in creation of really amazing array of colloidal structures ranging from opals or to a myriad of interesting inverse opals. In vertical deposition a substrate on which colloidal crystal is to be formed generally stands vertically in a dilute suspension of colloidal particles. Give the right conditions as the solvent from the suspension evaporates a face centered cubic colloidal multilayered structure is deposited. Vertical deposition over the years has been used with various types of particles, but the most work has been done using polystyrene and silica colloidal particles. Vertical deposition had been shown to reproducibly yield dry crystalline colloidal crystals with significantly

less defects under the right conditions. Some of the important studies have shown that higher crystalline quality can be attained using systems with low solvent surface tension and low ionic strength[66], high substrate wettability[67] chemical or thermal modification of the particles has also been shown to help[4, 5], and deposition on a tilted substrate has also been found to have a significant effect on the final crystal quality.[6] For deposition from water various researches have shown the optimal temperature ranges to be between 55 and 65^{o}C.[7] Various particle concentrations have been studied and they vary significantly from system to system. In our case we are using a significantly hydrophobic surface and that is one major aspect that goes against us as it will be seen in the results. This fairly simple yet elegant method will be used to grow colloidal crystal in the following sections, but the main aim will be to obtain a monolayer out of them, by removing the layers above.

3.3 Experimental

The idea here is to use appropriately functionalized particles and substrates in a vertical deposition experiment which is tuned to accommodate these changes and obtain a single monolayer by each run of vertical deposition. Then it was visions that by using a step by step approach we could form a multilayered colloidal crystal. However, as mentioned earlier in the introduction the focus of this chapter will be in obtaining a monolayer of good quality. The schematic showing the whole process for this experiment is shown in figure 3.1. In the following experimental sections protocols and recipes used to functionalize the substrate and particles will be discussed. All the chemicals used here were purchased from Sigma-Aldrich and were used without further processing or treatment, unless specifically mentioned otherwise.

3.3.1 Alkyne Functionalization of the Glass Substrate

The protocol used here for functionalizing glass substrate with terminal alkyne was same as the one used earlier in chapter 2. It was a two step approach, in which first the substrate was functionalized with amine, and then it was reacted with propargylchloroformate to obtain the necessary alkyne functionalization. This propargylchloroformate recipe was chosen over the one using 4-pentynoic acid because of simplicity of the process which eliminated multiple steps, including preparation of the buffer, and the fact that it had already been demonstrated to work in literature, though for particles but as from our previous experiments it has also been demonstrated that this recipe works for glass surfaces while sedimenting colloidal particles on it.[8] Another reason to use this is because of the higher yields this reaction offers when compared to the amide formation via the use of EDC as an initiator. So the following step a first covers the amine functionalization of the glass surface, and then step b describes the reaction with propargyl chloroformate.

a. Amine Functionalization of Glass Surfaces

For amine functionalization of glass slides one of the recipes studied by Youngblood et al.[9] was employed. Cut pieces of glass slides were cleaned using piranha etch (Sulphuric Acid and Hydrogen peroxide in 3:1) for 30 minutes, followed by a step involving thorough washing of the substrates with copious amounts of DI (Milli-Q) water and then the substrates were dried under a spray of nitrogen. A 25 ml solution of 1% 3-aminopropyltriethoxysilane (APTES) in ethanol was prepared, and the dried glass substrates were immersed in this solution in a Petri dish. The reaction was allowed to go on for 2 hours to form a uniform self assembled

monolayer providing terminal amines for further reaction on the surface of the substrate. Glass slides were then washed using copious amounts of DI (Milli-Q) water, followed by washing with copious amounts of ethanol to remove leftover unreacted silane molecules and then dried using a stream of nitrogen.

b. Reaction with Propargylchloroformate

Amine functionalized glass substrates were then immersed in a solution of 20ml dry acetonitrile (ACN) containing 600 µL of propargyl chloroformate and kept under inert conditions to react overnight. These glass substrates were removed next morning, washed with copious amounts of DI (Milli-Q) water, followed by copious amounts of ethanol and dried using nitrogen. These alkyne functionalized substrates were immediately used for the next step which in this case will be vertical deposition.

3.3.2 Azide Functionalization of the Particles

For this section we experimented with two types of particles. We started off by using our previously described 0.5 µm PS-PEG-Azide particles, and then moved on to use silica particles functionalized with azide. The recipe to functionalize PS-COOH flurospheres (Invitrogen Inc) is described in the part a. of this section and the new recipe for making silica-azide particles is described in the part b.

a. Azide Functionalization of PS-COOH

Carboxyl modified 0.5 µm PS particles with fluorescent cores (fluorospheres) by Invitrogen Inc. were used for these experiments and a hetero-bifunctional PEG oligomeric spacer (as previously shown in figure 2.1) was used to provide with the necessary azide functionality on the surface. To functionalize PS-COOH fluorophores N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide - hydrochloride (EDC) was used as an activating agent, to activate the surface bound terminal carboxylic acids on the PS particles, to form an amide bond with the amine ends of O-(2-Aminoethyl)-O′-(2-azidoethyl) nonaethylene glycol, an oligomeric hetero-bifunctional PEG. 50 µL carboxyl functionaized 0.5 µm PS (2% solids) particles were dispersed in 1mL of DI (Millipore) water. Centrifuged at a rate of 13.2k rpm for 20 minutes and supernatant was removed, in order to get rid of the original suspension it comes in. Re-dispersed in 700 µL MES buffer solution (50mM) at PH 4.6. The bifunctionalized PEG had amine at one terminal end and azide at the other, with ten repeat units of the ethylene glycol. 50µL of this amine-PEG-azide was added to the dispersion of particles in MES buffer (60 µM), followed by addition of 10 µL of 5M EDC (6.67 mM) solution prepared in MES buffer at PH 4.6, and left on vortex mixer set on slow for 30 mins. After initial 30 mins another 20 µL of 5M EDC solution in MES was added, and the solution was left to react on vortex mixer for another 30 mins. Centrifuged after a total reaction time of 1 hour, at 13.2k rpm, for 20 mins and supernatant was removed. Redispersed in DI (Millipore) water, followed by another step involving centrifuging it at 13.2k rpm, followed by redispersion in DI to remove unreacted reactants.

b. Functionalization of Silica Particles with Azide

The protocol used for functionalizing Silica particles with azide was in part adopted from a similar approach taken by Brittain et al.[10] however, changes were made to suit our requirements. This process involves a two step approach, where first the particles are functionalized with bromide functionality and then this bromo functionality is swapped with an azide one using a SN_2 type nucleophilic substitution using sodium azide. This approach is shown in the figure 3.2 as adopted from Brittain et al.[10] The two steps process is discussed below.

For this experiment various sizes of lab grown silica particles were used. It was made sure that the particles were dried and had as less as possible aqueous content on its surface. For this they were heated up first in incubating oven at around 70^{o}C for overnight, and then dispersed in anhydrous toluene. These particles were then centrifuged down, out of the toluene at 3000 rpm for 30 minutes (for 500nm silica particles) and supernatant removed. Redispersed in fresh 45ml anhydrous toluene, and centrifuged down using similar conditions, supernatant was removed, and these cleaner particles were now ready for the following functionalization reactions. The recipe mentioned here is for 500nm particles as used in a number of experiments, however, it was also used for 400nm particles, with slight modifications in the centrifuge settings. Rest of the recipe was kept the same.

Functionalization of Silica Particles with 3-Bromopropyltrichlorosilane

These cleaned particles (in a typical batch usually around 0.5 g to 1g in amount) were then redispersed in 20ml of fresh anhydrous toluene in a two necked-round bottom

flask, and the flask's openings were tightly closed using septa. These particles were sonicated for at least an hour or till they were well dispersed. Once dispersed this flask was moved to an oil bath preset at 80^{o}C under the hood, purged with nitrogen using a steady stream via inserted needles in the septa, while it otherwise remained sealed to maintain inert atmosphere. After purging it with nitrogen 25ml of fresh anhydrous toluene was added, and the flask was sonicated once more for 10 minutes. After second sonication it was moved back to the oil bath, and 5mL of 3-bromopropyltrichlorosilane in 15mL of toluene was added dropwise. The reaction was allowed to go on for 18 hours, at the same temperature in the oil bath. Then the particles were centrifuged out at 3000rpm for 30mins. Redispersed in toluene and centrifuged out again. This was repeated at least 5 times, and then the particles were redispersed in DMF instead of toluene without drying.

The particles were centrifuged out of the DMF at 3000rpm for 30 minutes, and then redispersed in 45ml of DMF again twice, followed by centrifuging it down to remove toluene or any other unreacted species from the last step. These particles centrifuged out of the last step were immediately used for the next step without drying or any other intermediate steps.

Azide functionalization by SN_2 Nucleophilic Substitution

These 3-bromopropyl-functionalized silica particles were then redispersed in 20ml of DMF in a round bottom flask. The dispersed particles were moved to an oil bath preset at 80^{o}C under a hood, and stirred for 10 minutes, followed by addition of 80mL fresh DMF, and addition of 2 g of NaN_3. The flask was propped close with the septa,

and the solution was stirred at these conditions for 18 hours. Once the reaction was done, the particles were recovered by centrifuging it out at 3000rpm for 30 minutes. These centrifuged down particles were then redispersed in ethanol and centrifuged out again using same conditions, and the supernatant was removed. Followed by a redispersion in water, to remove the salt and unreacted species, and then centrifuged down again using same conditions and the supernatant thrown out. This simultaneous re-dispersion and centrifuging in ethanol and water, with removal of the supernatant to remove the impurities were done for 3 times each, for ethanol and water, and in some cases more than that if the particles were still found dirty upon characterization in SEM.

3.3.3 Vertical Deposition

As discussed in the previous section that vertical deposition has become pretty standard and extremely reliable method for making colloidal crystals and, opals, so now here in this section an approach will be discussed as to how we can incorporate a chemistry like Cu(I)-catalyzed azide alkyne cycloaddition in this whole process. The idea is to use the ‘click-able’ functionalized substrates and appropriately functionalized particles, which in our particular case for these set of experiments are either PS-PEG-azide particles or Silica-Azide particles and glass cover slips or sliced microscope slides as mentioned where used, functionalized with alkyne using the propargylchloroformate route.

The fairly basic and extremely simple process of vertical deposition was used in several different ways during the course of these experiments. The idea was to find the sweet spot of conditions in which we can form a good quality colloidal crystal that would eventually lead to a good quality monolayer. It is pertinent to notice here that these

surface chemistries involved here are widely different from what has been previously used in vertical deposition systems. So the whole process was carried out as more of a smart trial based approach. The standard vertical deposition conditions used widely over the course of these experiments, were that these particles (if silica-azide) were dispersed in ethanol, and then were deposited out of ethanol using 45^{o}C temperature. The substrates that were eventually found to work better were sliced microscope glass slides functionalized with alkyne. The common aspect of almost all the vertical deposition steps was that the crystal was kept wet, and not allowed to dry. This was accomplished by not letting the crystal grow completely to the edge of the substrate, and hence small time intervals were chosen for the vertical deposition. Ranging from 2 hours to 5 hours, for a silica-azide system vertical deposited out of ethanol we got varyingly different results. These results are discussed below in the next sections along with the discussion, and each of the vertical deposition steps employed are also discussed there. However, to summarize the general outline of the protocol for vertical deposition, it was carried out of the ethanol at an elevated temperature ranging from 35^{o} to 45^{o}C, in an incubator, covered completely to reduce atmospheric disturbances caused by air flow, and the ethanol not allowed to evaporate completely so that the colloidal crystals are still partially wet when removed. This was necessary as it was observed that when the colloidal crystal is allowed to grow completely and hence it dries up, it becomes harder to wash of the upper layers above the monolayer without destroying or delaminating the monolayer along with it. This is partially attributed to the capillary forces involved in forming the colloidal crystal as the solution (ethanol in this case) evaporates. The dried crystal is harder to manipulate especially when the aim is

to remove the upper layers to obtain the monolayer. It is also important to note here that mostly vertical deposition was carried out in an incubator using Nalgene's polyethylene vials, unless mentioned otherwise.

3.3.4 Cu Catalyzed Click Reaction and Removal of Colloidal Layers

Once the colloidal crystal was obtained, and it was removed wet from the vials, it was immediately moved to the Petri dish containing catalyst solution. The standard catalyst solution as used previously of 10mM $CuSO_4.5H_2O$ and 50mM L-Ascorbic acid was used. The click reaction was allowed to run for 5 hours, and then the colloidal crystals were immediately moved to a 5M Tween-20 solution to remove the layers above the monolayer, while shaking it on belly bottom shaker for overnight. This step is of great importance as it will be seen in the results that it is actually hard to obtain a nice and clean monolayer. Several different approaches were tried and are discussed in the results below. The colloidal crystal obtained after this was moved to a Petri dish containing water, to remove the Tween-20 solution and if there are any remaining species from the previous steps. Then the substrates were thoroughly cleaned in water, followed by cleaning with ethanol gently to remove the unwanted remains of the upper layers of colloidal crystal or the chemicals used in the synthesis above. After this the colloidal crystal on substrate was dried and taken for SEM or optical microscopy as discussed below in the results.

3.4 Results and Discussion

In this section results are discussed in a progressive sequence, which is also incidentally setup chronologically with each next set of experiments resulting in either improvements or new findings. The aim for these experiments was to obtain monolayers, which was achieved; thought the quality of those monolayers can be greatly improved. A whole set of experiments was performed to improve the quality of the monolayer, however, with less improvements achieved, more findings were made regarding what might not be working fine, and in which direction the improvements can be made.

In the following set of sections each, a different group of experiments is discussed, where the sections are divided based on some common theme or aspect in all of these experiments.

3.4.1 PS-PEG-Azide Particles for Vertical Deposition

These were some of the very first experiments performed, in which vertical deposition was explored as a suitable way for creating colloidal crystals that can eventually give monolayers for subsequent processing.

The particles used were PS-PEG-Azide, made as discussed previously, and the substrates used were microscope cover slips (22x22 mm) functionalized with alkyne as described previously using the propargylchloroformate recipe. This set of vertical deposition (VD) experiments were carried out in an incubator set up at 55^{o}C, 0.1% of the particles by volume particles were dispersed in DI water, as it is fairly standard for water to be used for making PS opals. The first few experiments were allowed to run

completely overnight till the water evaporated completely, in order to observe the quality of the crystal grown, and it was observed that the quality of the crystal was very bad. The crystal formed was multilayered, and vertical deposition was not able to form a continuous crystal as it can be seen in figure 3.3. For comparison a colloidal crystal made out of PS-COOH on a bare piranha cleaned substrate is shown too in figure 3.3. In the following figure 3.4 it can be seen that optical micrographs of the colloidal crystal show poor quality, and stripped nature along with its multilayered structure. Now this effect can be because of the particle's surface functionalization as well as the substrates surface. SEM images of these crystals show a bad quality colloidal structure which can be seen in figure 3.5 and these results check out well with the observations made in the optical micrographs. These bad quality crystals would lead to imperfect monolayers as it will be observed in next few experiments. For this particular experiment the cleaning and removal steps were not carried out.

However, it will be observed in the next experiments that the hydrophobic nature of the alkyne substrate proves to be a great hindrance in terms of forming good quality crystals, as particles can be adjusted by varying the solvent used in the VD. Several experiments were performed using this system with bad quality opals obtained in results, and so it was decided to move on to a new system in which silica-azide particles could be used. The main reason to move to silica-azide was that silica opals are conventionally made by using ethanol as a solvent, and it was hypothesized that an improvement can be made in the crystal quality by making the process faster. That is if the evaporation rate is increased, maybe instead of getting these stripes we would get a continuous film, as the meniscus would flow all along the substrate surface rather than

jumping down like it does in this case. However, PS-PEG-Azide particles were not stable in ethanol, and so a move to Silica-aizde particles was considered. Next set of experiments are performed using silica-azide particles.

3.4.2 Silica-Azide Particles for Vertical Deposition

These set of experiments were performed using silica-azide particles which were made using the same two step recipe discussed above in the experimental section. The substrates used were microscope slides cut in small 12mm x 25mm sections and functionalized with alkyne using the recipe discussed in the experimental section. This move to microscope slides was based on the fact that in other experiments in our group we were getting better results with use of microscope slides for making opals instead of using the thin cover slips, and a factor suspected to play some role was the relative thickness difference between cover slips and the microscope slides.

These silica-azide particles were stable in ethanol as it is also seen in the recipe that they are centrifuged out of ethanol and redispersed in it several times. This fact allowed us to use ethanol for vertical deposition. So the recipe followed for vertical deposition in case of these particles was same as the one discussed in the experimental section. Several different concentrations were used and will be mentioned along with the individual results. In some experiments higher temperatures were used and instead of incubator heating oven was employed.

a. Silica-Azide Colloidal Crystal and Monolayer from a Heating Oven

The particles use for this set of experiment were 400nm Silica-Azide particles prepared according to the recipe mentioned above in the experimental section and the substrate was microscope glass slides functionalized with alkyne as mentioned above. The concentration of the particles in ethanol use for these experiments was 3.6% by weight. The very first encouraging results obtained were result of an experiment run in a fashion which can be at best described as a trial. This experiment was performed under circumstances that required dire measures as all of the incubators in lab were already in use and the particles and substrates prepared following a procedure that took over two days were ready. As per the common practice it was deemed detrimental to wait, as the substrates for these experiments are always used fresh out of the Petri dish, so the experiment was carried out in an incubator oven conventionally employed in out lab for incubating and drying glass ware. The temperature fluctuations were large, ranging from 50^{o}C all the way up to 75^{o}C, and so the first trial resulted in the observation that plastic nalgene vials were not a good choice for this experiment at these temperatures as one of them just gave way and melted on the aluminum foil placed on the base of the oven. For the next trial a 20ml glass scintillation vial was used with two set of substrates placed in a manner showed in figure 3.6. The vial was covered by a large size empty dish inverted upside down to reduce the effect of outside air flow, as that has been previously shown to result in bad quality opals. In an hour it was observed that the ethanol solution was about to evaporate complete, and so the substrates were immediately pulled out while they were wet, and placed in the Cu catalyst solution for click reaction prepared as described in experimental section above, and let to react for 5

hours. While one of the samples (shown in fig. 3.7 and 3.8) was kept for SEM after this click reaction step, the other one was washed in DI water by shaking on bellybutton shaker overnight to produce monolayers. Note the use of DI water here, as the Tween-20 was not used for this step, as previously good results were not observed with the use of Tween-20. However in the future experiments use of Tween-20 will once again be investigated, and with adjustment of time good results will be obtained. Figures 3.7 and 3.8 show top view of the colloidal crystals obtained as a result of these experiments and this was the first sample which was not washed in DI water on a bellybutton shaker for overnight. Figure 3.9 show the cross section of this same crystal, and it is easily observable that we have a huge crystal formed with over 100 layers formed above the monolayer.

The sample which was cleaned over night in DI water while rotating on a belly button shaker showed significant signs that the upper layers were being washed away. We observed several regions with monolayer, and signs of the remains of the secondary and tertiary layers above the monolayer were also visible as shown in Figure 3.10. This result was encouraging and showed that monolayer can be obtained by incorporating click chemistry in the process of vertical deposition so that the first layer is anchored to the substrate via 1,2,3-triazole link formed as a result of the CuAAC reaction. Better regions showing monolayer can be seen in figures 3.11 and 3.12. The cross-section view of the monolayer can be seen in the figure 3.13.

These results were very encouraging and so in the next section we further investigate the process of vertical deposition to create better quality monolayer.

b. Silica-azide Click Chemistry Assisted Colloidal Crystal and Monolayer from incubator

In this section the normal recipe and protocol used by our group for making opals and colloidal crystals was employed and so an incubator was used instead of an oven. The attempt was to improve the monolayer quality and create better colloidal crystals. The general protocol followed for this set of experiments used the same silica-azide particle and alkyne substrate preparation recipes as discussed in the experimental section. The particles used for these experiments were 500 nm silica particles, instead of the 400 nm used in the last section. The significant differences between these experiment and the last ones were in the fact that incubator was used for these, the temperature used was 45^{o}C, the colloidal crystal was grown for 1 hour and 30 minutes and substrates were pulled out wet, the particle size was different that is 500 nm, and the post washing steps involved twirling of the substrates on a belly button shaker in 5M Tween-20 solution overnight, then washed in DI and observed in an SEM. Several orientations (angles) of the substrate relative to the receding meniscus of the ethanol particle solution were studied and important conclusions were drawn.

In figure 3.14 the relative position of the sample and polyethylene Nalgene via is shown for the next couple of results discussed. This slanting angle of the vial was adopted after a couple of attempts lead to very bad quality of colloidal crystals with the vertically straight vial and slanting substrate. However, it can be seen in figure 3.15 that the resulting colloidal crystal was not of such a good quality either. This resulting bad quality was attributed to the fact that the substrate was very horizontal and this could have lead to a large number of particles just being sedimented along with the particles left by the evaporating front of the ethanol. As it is known that sedimentation gives

relatively bad quality opals when compared to the ones obtained by vertical deposition. After washing away the upper layers a monolayer was obtained as it can be seen in figure 3.16, which was however of not such prime quality, as the surface coverage was also low. In figure 3.17 a region of multiple layers can be seen along with the monolayer, this shows that though Tween-20 solution is washing the layers away, it is not very effective in washing away the upper layers and in fact is just as good as DI water.

Schematic in the Figure 3.18 shows another orientation of the substrate and vial studied for making colloidal crystals. It can be seen in Figure 3.19 that the resulting monolayer was also not of great quality, and in fact the surface coverage went even lower. This was also visible in the colloidal crystal as it was thinner and was of bad quality itself. Figure 3.20 shows the closer image of the monolayer and the region where remaining unwashed secondary layer can be seen. It can be observed here that the crystal quality of the monolayer here is better when compared with figure 3.16, which was for the different vial and substrate orientation, and hence this also points towards the fact that sedimentation would have played a role in the previous set of results shown. This rises important questions about the effect of crystal quality on the crystalline quality of the monolayers obtained, and it seems like that initial crystal quality plays a huge role in terms of giving better quality monolayer. It can be said that if the crystal quality of the opal or the colloidal crystal is not good then we can result in a poorer monolayer, which might have huge defects and lower surface coverage.

This is an important question and can also be asked from another perspective by comparing two different sizes of the particles used, as shown in the figure 3.21, that 400

nm silica-azide particles generally formed better crystals than the 500 nm silica-azide particles on the same substrate. Which is no surprise based on our group's previous work that shows, that it gets hard to obtain good crystal quality opals when larger size silica particles are used.

Keeping these results in view a set of important conclusions and recommendations are drawn, which are discussed in next section.

3.5 Conclusions

In this chapter the set of experiments shown and discussed give important results that help us conclude that CuAAC click reaction can be incorporated in a colloidal assembly procedure like vertical deposition to obtain monolayer. This significant step is of vital importance if multilayered colloidal crystals are to be formed in a directed fashion using click chemistry. These set of experiments provide with enough proof to show that monolayers can be obtained by using functionalized silica-azide particles on an alkyne substrate, however the quality of these monolayers needs to be greatly improved before a secondary layer or a monolayer on top of the first one is planned to be made. These experiments also help draw important recommendations, like PS-PEG-Azide particles are not stable in ethanol and they do not give good quality crystals when opals are attempted to be made using vertical deposition. The other important observations made include the effect of particle size, where just a difference of 100nm makes a huge impact on the crystal quality. Therefore in light of these experiments it is recommended that smaller particle sizes be employed for further studies of the kind. The click chemistry seems to be working; however a sweet spot of conditions still needs to be found when it comes to a technique like vertical deposition

which is more of an art if one needs to make good quality crystals. With each change in parameters the final results can be drastically different. It is also important to mention here that the hydrophobic nature of the substrate does play a detrimental role as far it vertical deposition goes as it can be seen in the stripes formed by the PS-PEG-Azide particles, and it might also be partially responsible for bad crystal quality in the silica-azide experiments, which result in a bad quality crystal. Click chemistry being functional it is also recommended that other approaches to colloidal assembly must also be studied, specifically the ones that aren't affected by the hydrophobicity of the substrate much like the flow cell method.{{90 Park,S.H. 1999}}

3.6 Figures

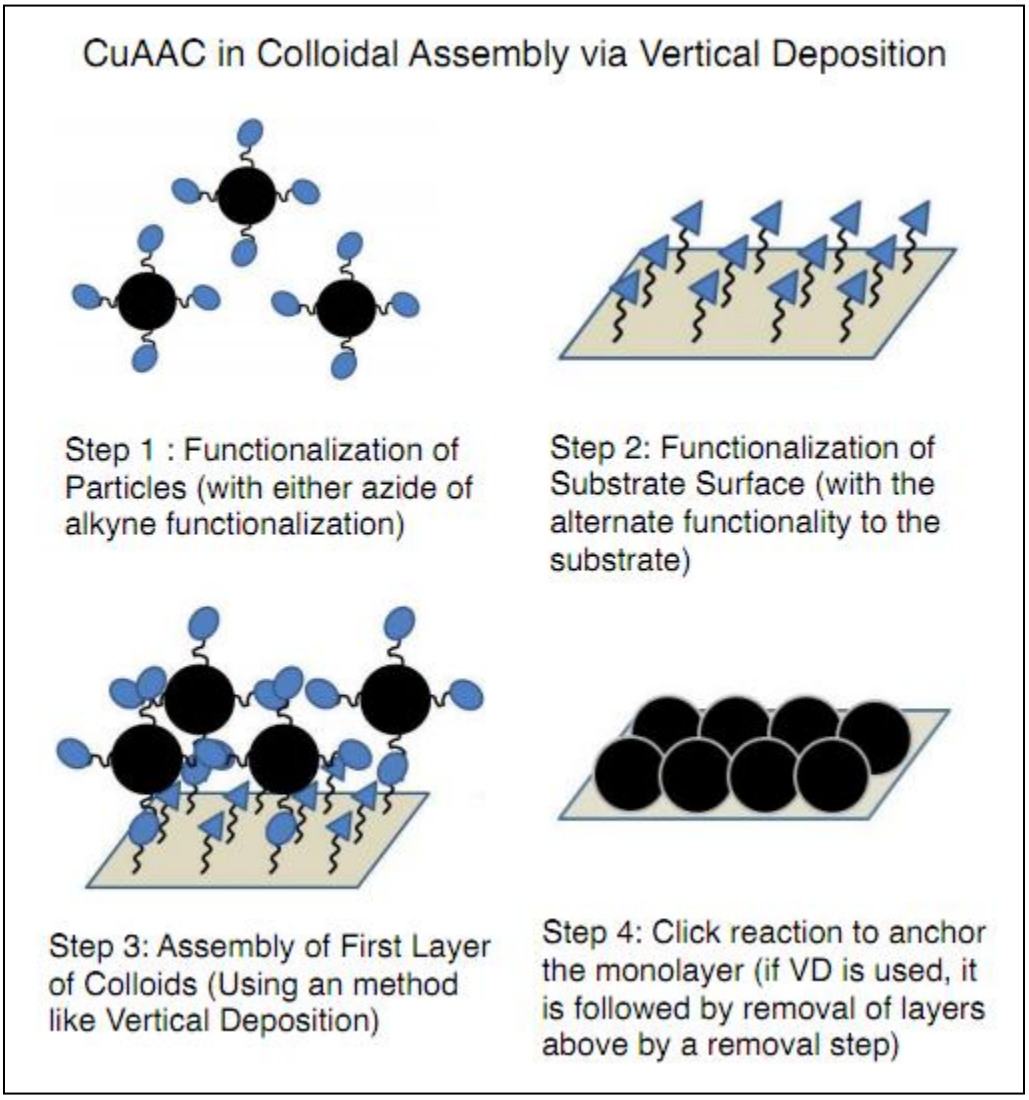

Figure 3.1 Schematic showing a general approach to obtaining a monolayer of colloids using functionalized particles and substrates.

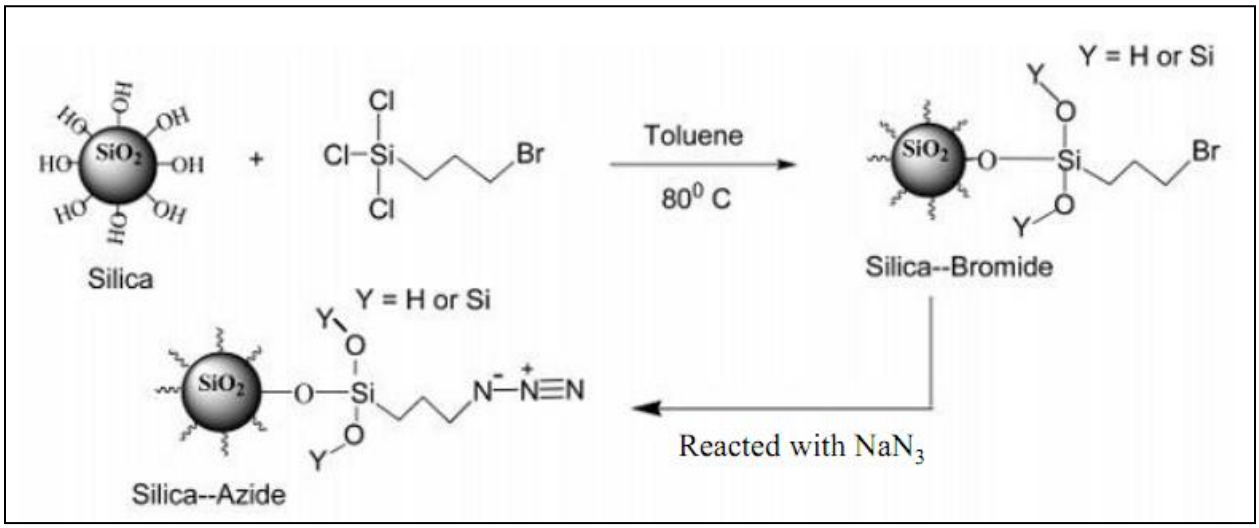

Figure 3.2 Silica particles functionalized with azide in a two step approach. Image adopted from Brittain et al.[10]

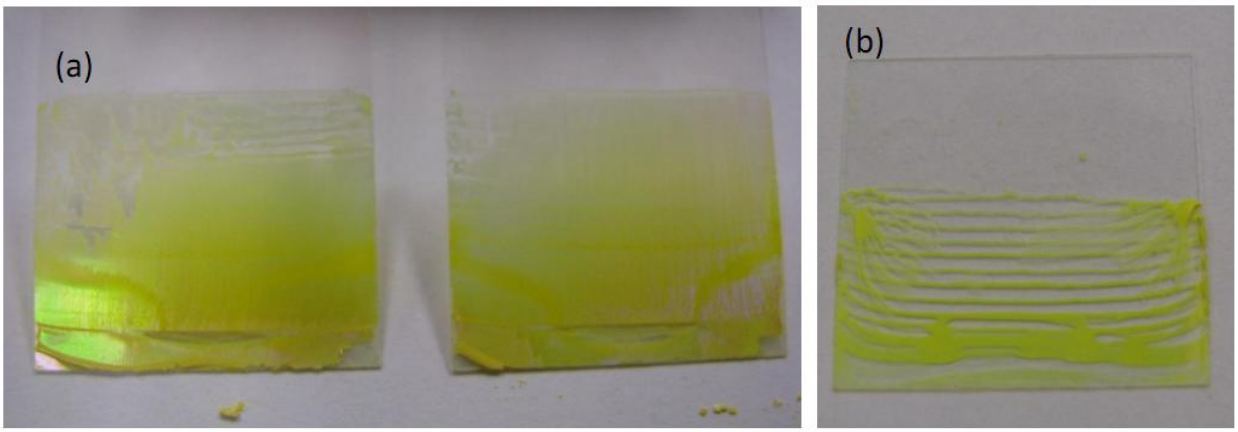

Figure 3.3. Photographic images of (22x22mm : for scale) microscope coverslips used for making colloidal crystals, and from comparison it can be seen that a) PS-COOH forms a continuous opal, whereas b) PS-PEG-azide forms a striped structure that is of poor quality. It should also be noted that in a) a piranha cleaned bare substrate was used and in b) a glass-alkyne substrate was used.

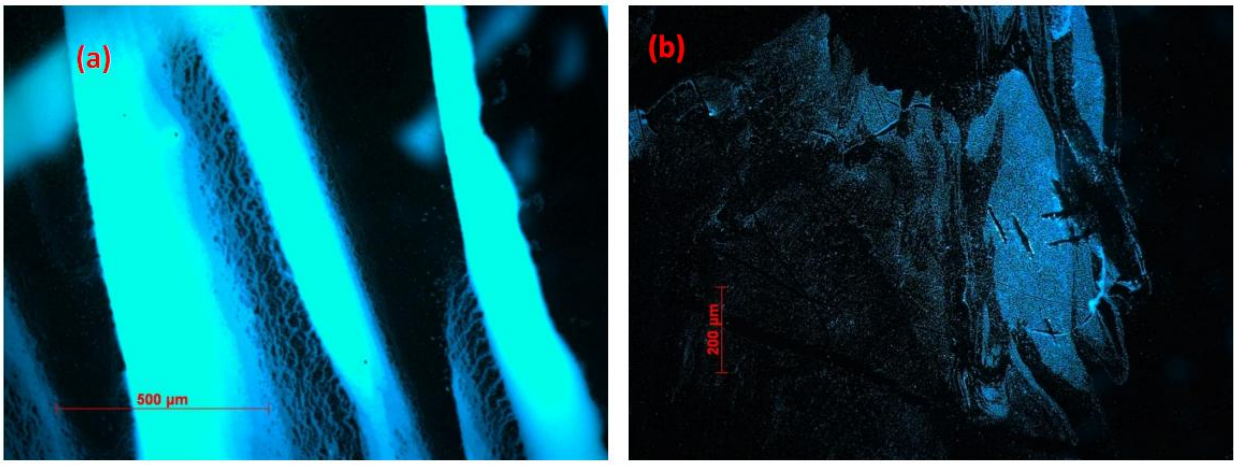

Figure 3.4 Optical micrographs of PS-PEG-Azide opal formed on a cover slip functionalized with alkyne. It can be seen here that a) the crystal formed is multilayered, and b) it is of bad quality as the opal is not spread continuously. This was also observed in forms of the stripes in figure 3.3 b.

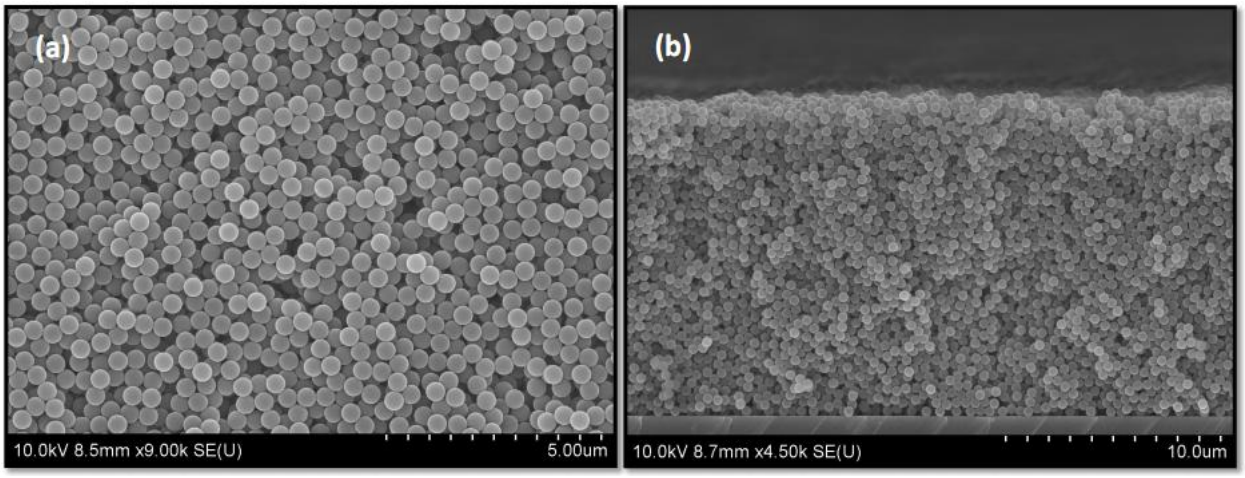

Figure 3.5 SEM images of PS-PEG-Azide opal formed from .1% by volume particle solution in DI water formed at 55^{o}C, it can be seen in a) top view that the crystal quality is very bad, and b) from the side view that it is a really thick multilayer colloidal structure, lacking periodicity characteristic of a colloidal crystal.

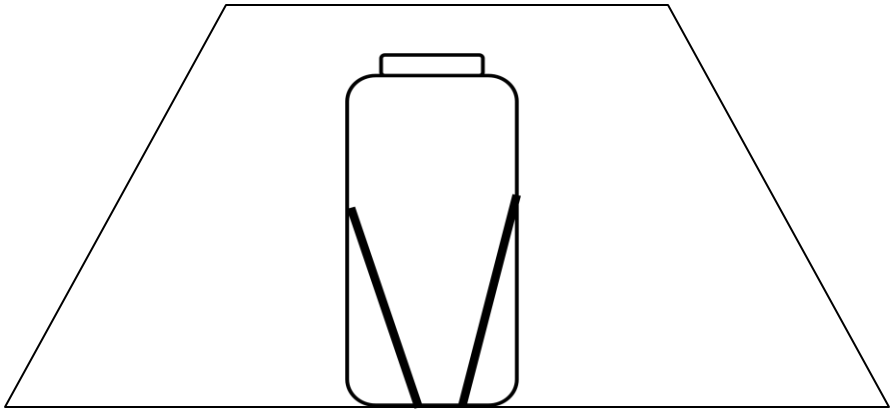

Figure 3.6 Schematic representation of the scintillation vial and samples' relative position and angle, placed in an incubating oven.

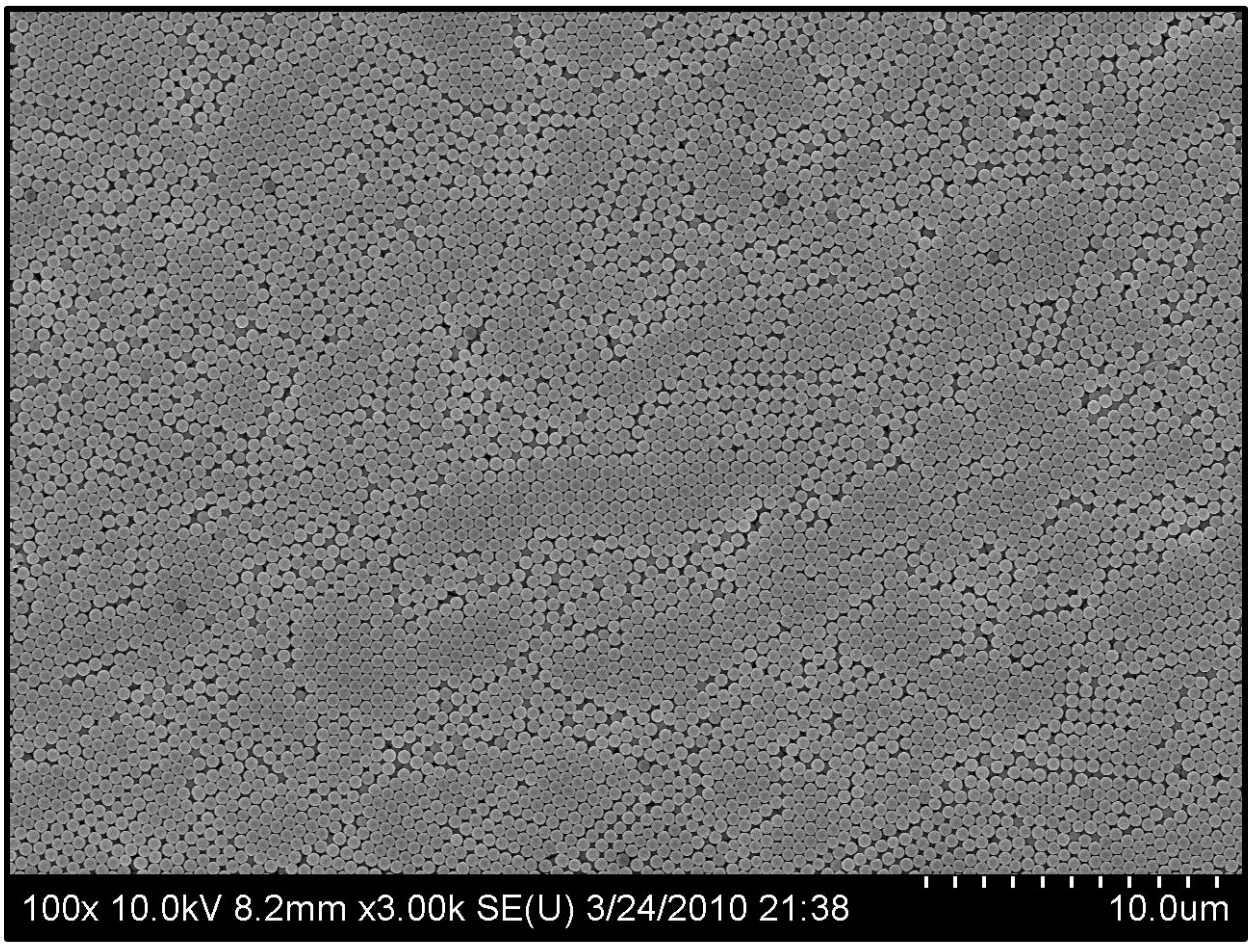

Figure 3.7 SEM of the colloidal crystal obtained using silica-azide particles on alkyne functionalized substrate in an oven.

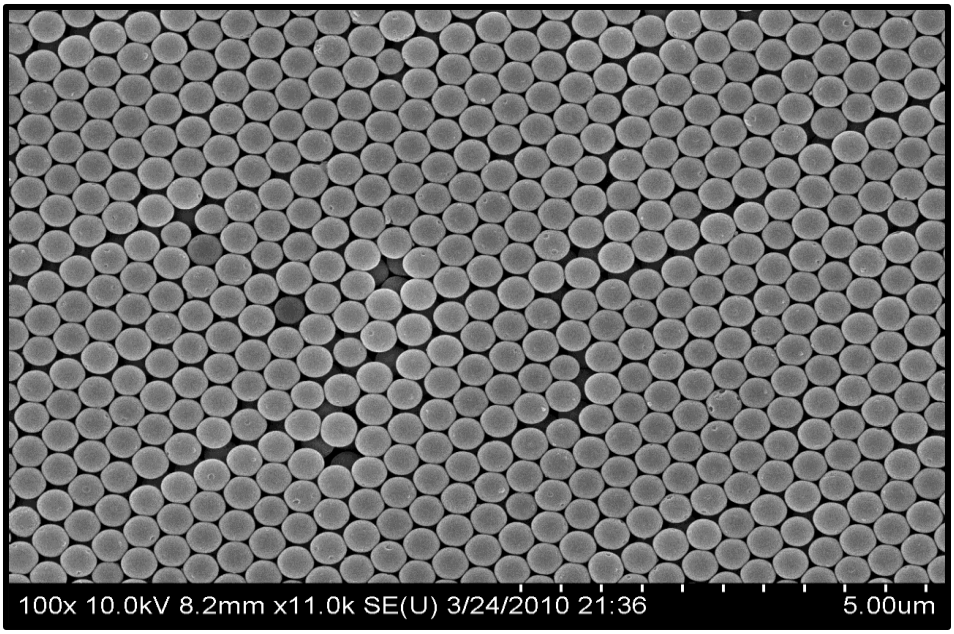

Figure 3.8 SEM image close up of the silica-azide colloidal crystal formed on the alkyne substrate formed showing a good crystal quality region.

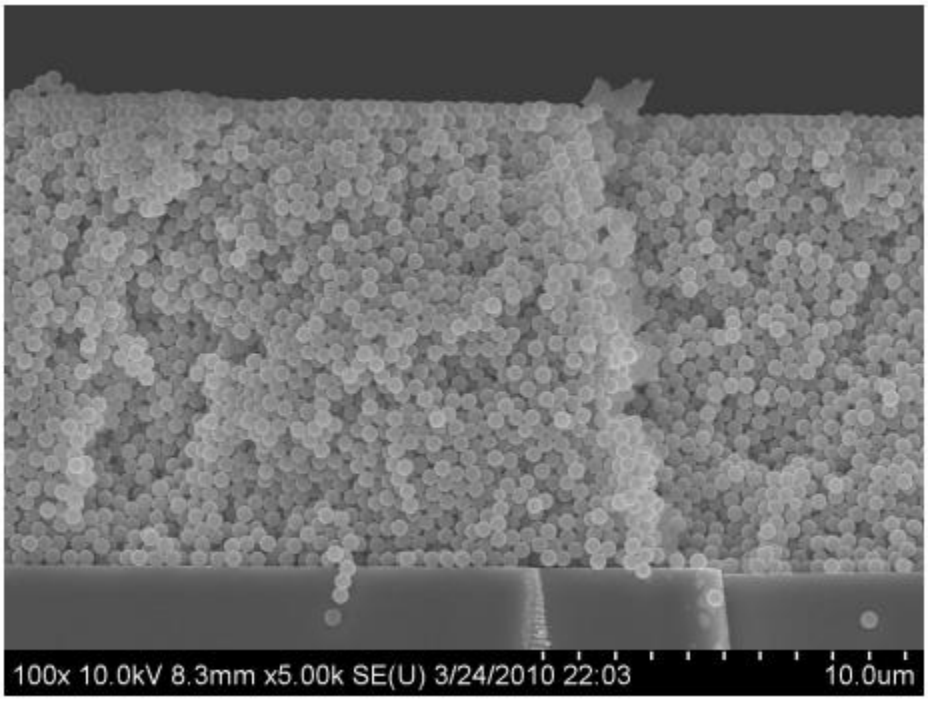

Figure 3.9 SEM Cross section of the same sample showed in figures 3.7 and 3.8, showing multilayered structure with over 100 layers formed above the monolayer.

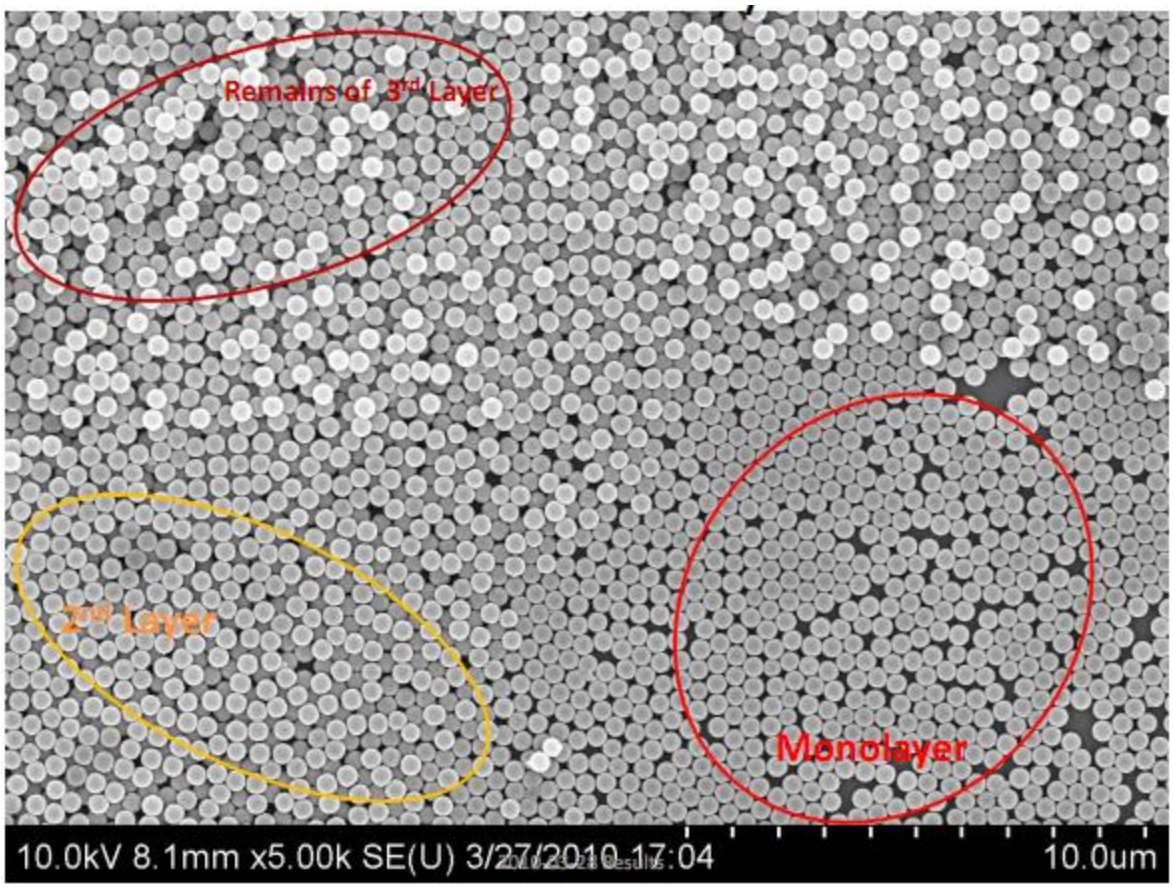

Figure 3.10 SEM of the washed colloidal crystal showing monolayer region, along with the remains of the multilayers removed. This shows that the cleaning is working and moreover that monolayers can be achieved using vertical deposition.

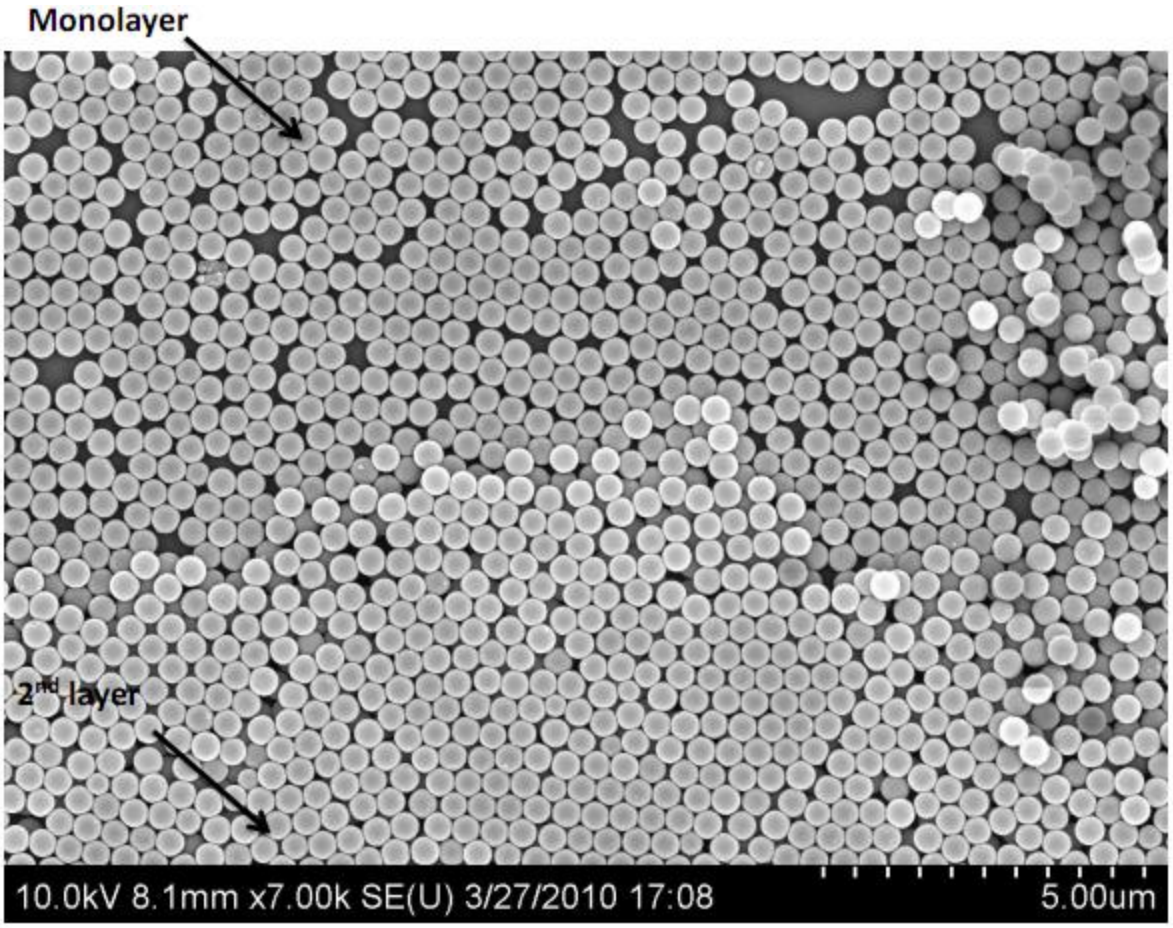

Figure 3.11 SEM image of colloidal crystal washed down to produce monolayer, also showing remaining parts of the second layer.

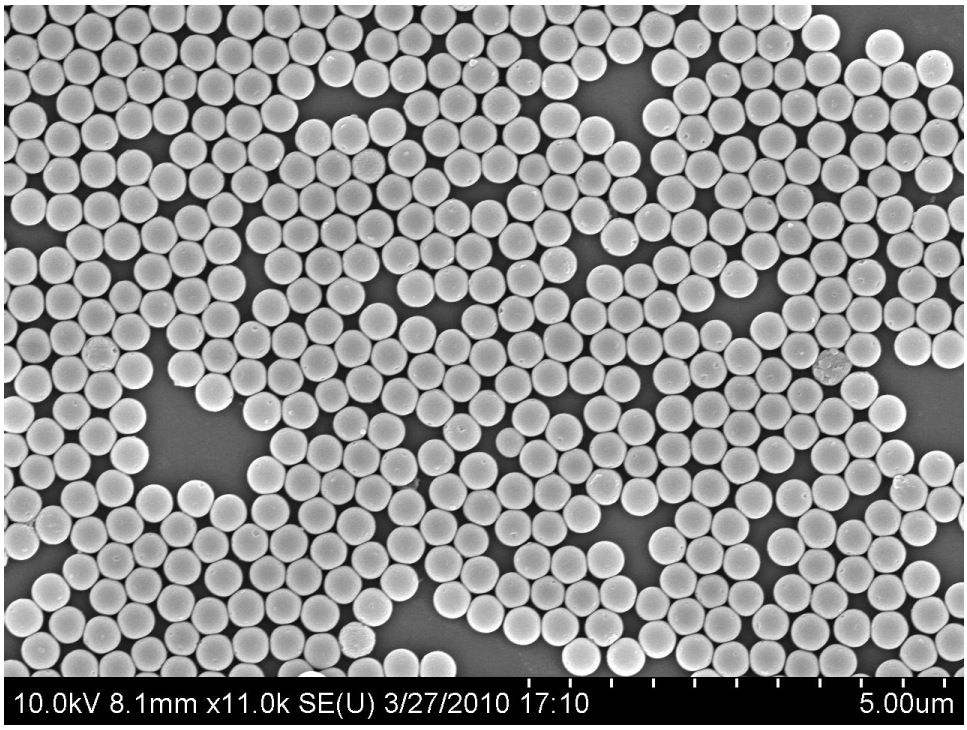

Figure 3.12 SEM of monolayer obtained by using click chemistry in vertical deposition followed by removal of layers above the monolayer.

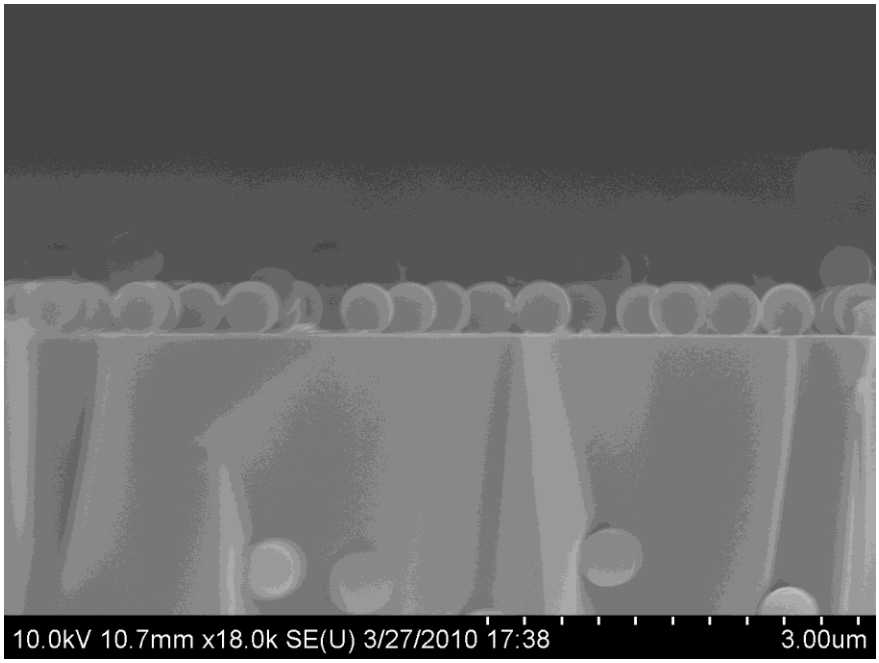

Figure 3.13 SEM cross-section view of the monolayer formed by using click chemistry in the vertical deposition.

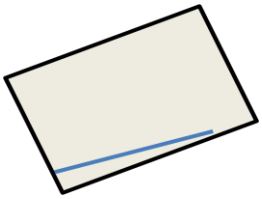

Figure 3.14 Relative position of the substrate shown in the vial which was held at a slanting angle instead of holding it straight, as it was observed that more dense crystals are obtained by doing so.

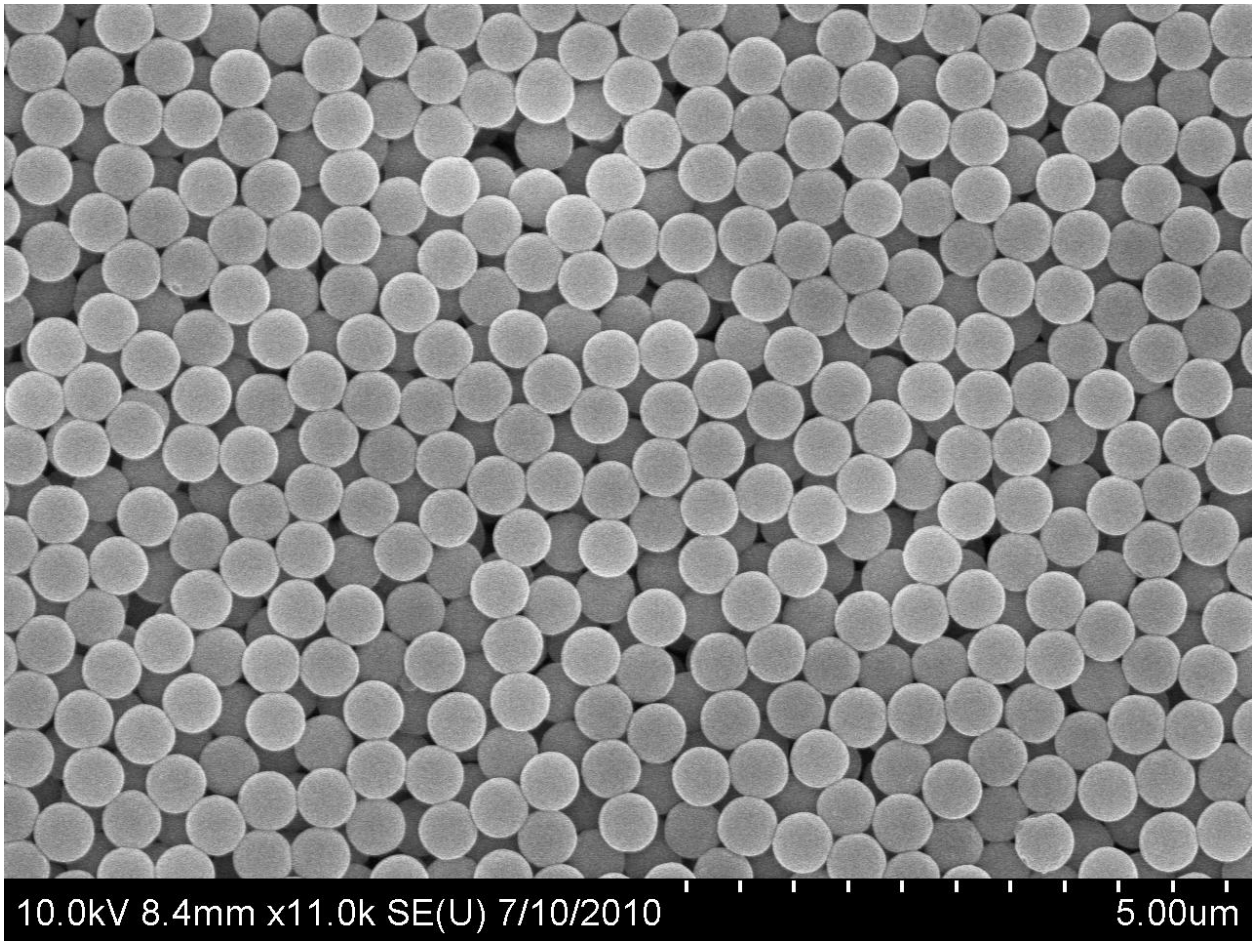

Figure 3.15 SEM top-view image of Silica-azide opal formed, showing bad crystal quality.

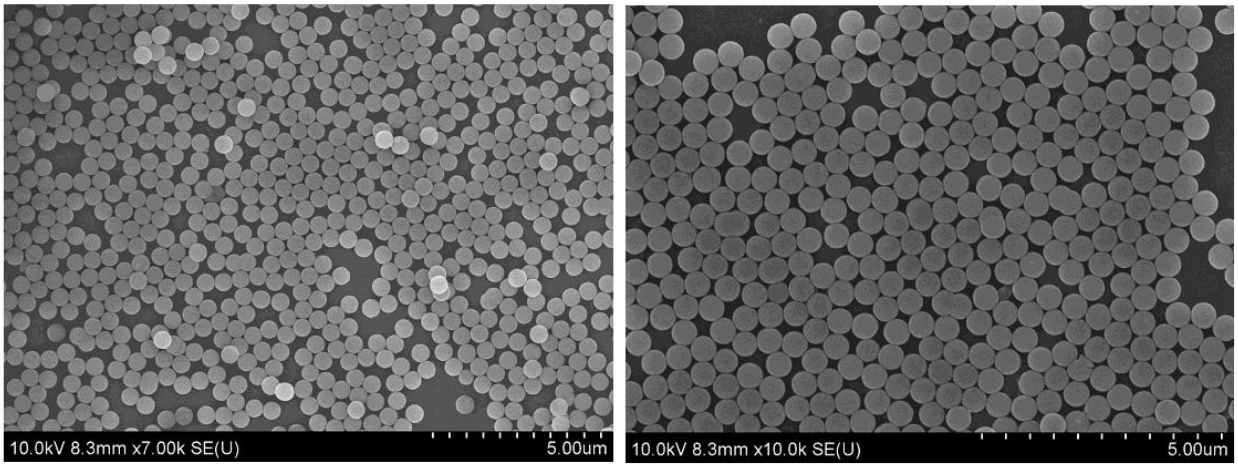

Figure 3.16 SEM top-view images of monolayers obtained after washing away of upper layers in Tween-20 solution overnight.

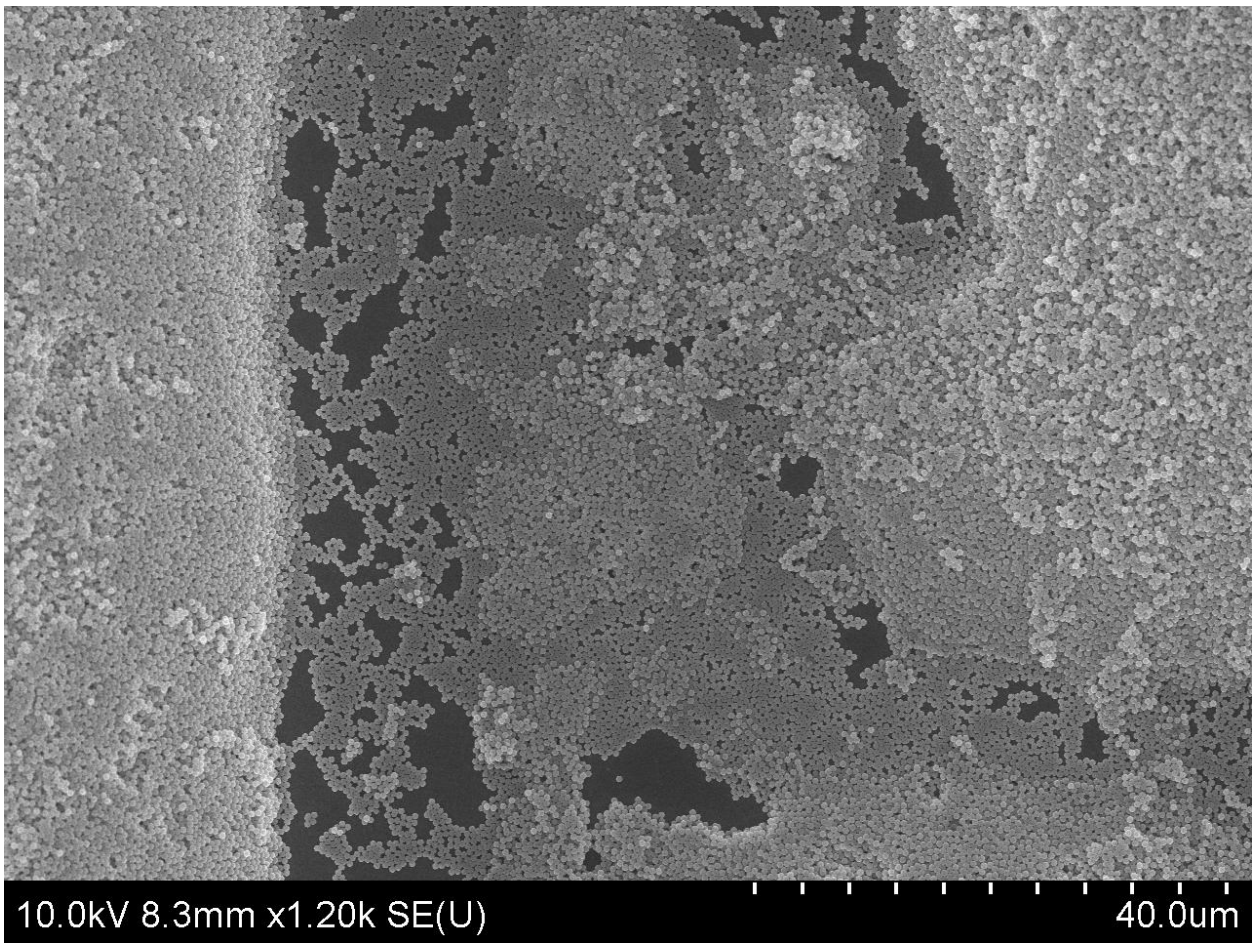

Figure 3.17 SEM top-view image of a region showing multi-layers and bad quality monolayer region.

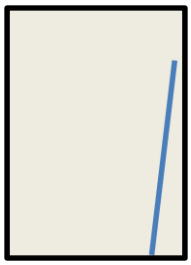

Figure 3.18 Schematic representation of orientation of the vial and alkyne substrate in the incubator.

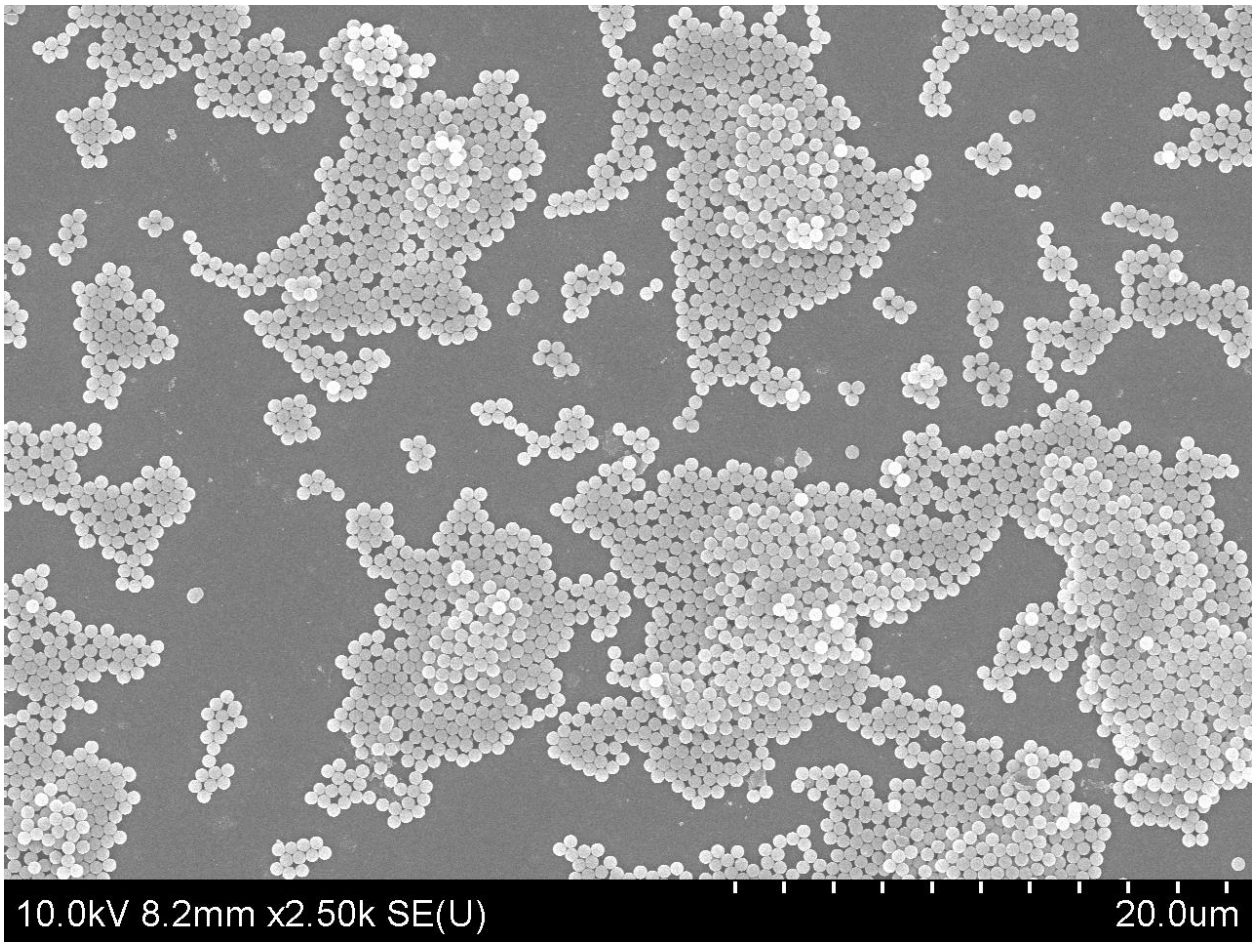

Figure 3.19 SEM top-view of monolayer and remains of the second layer obtained by click assisted colloidal assembly using an incubator with the substrate and vials relative positions shown in figure 3.18.

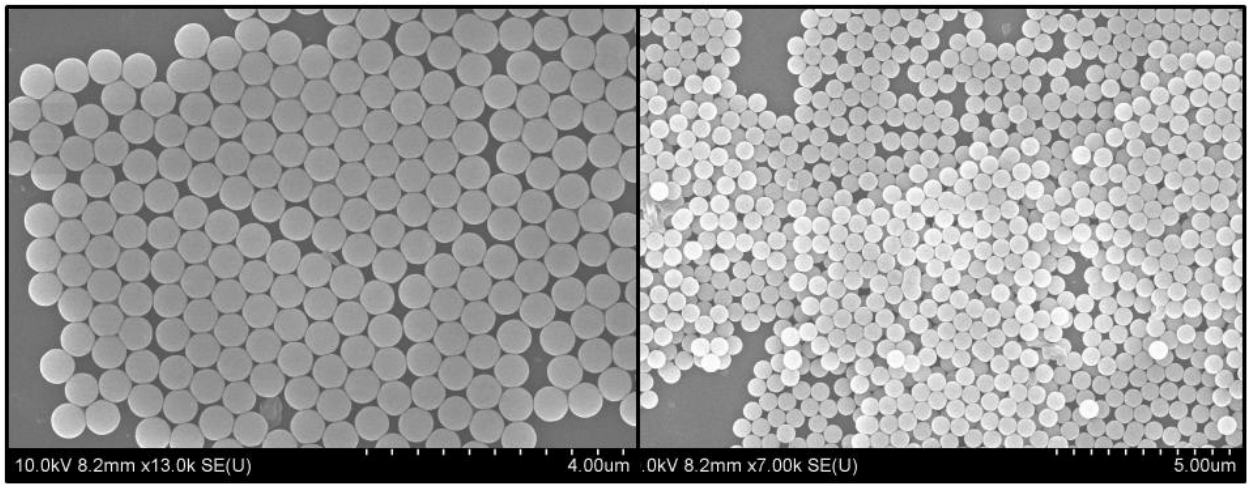

Figure 3.20 SEM top-view images of the monolayer (left) and two layered regions (right), where the right hand side shows incomplete washing away of the secondary layers, and the left side shows better crystal quality of the monolayer.

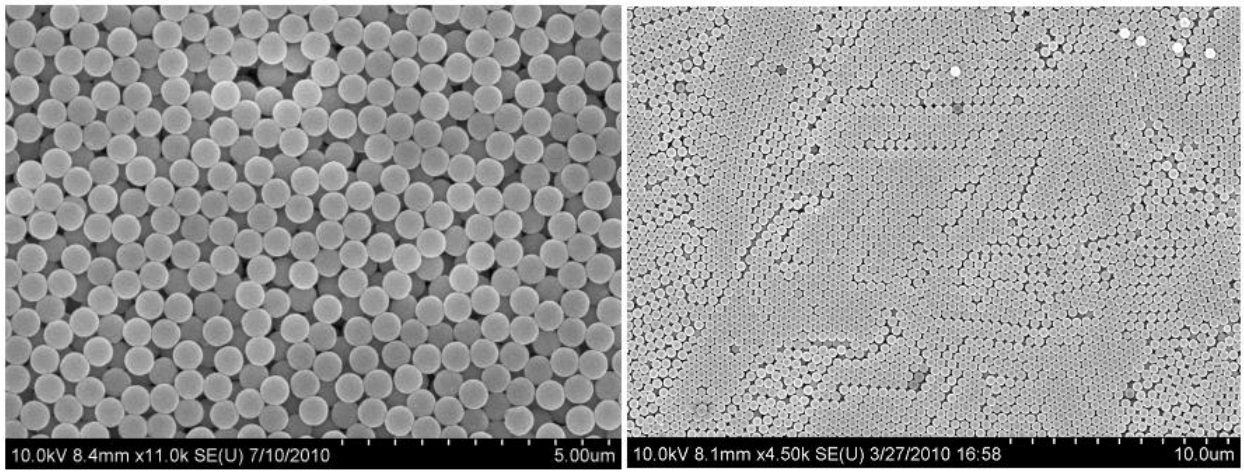

Figure 3.21 SEM top-view images of opals made using 500 nm Silica-azide particles (left) and 400 nm Silica-azide (right) prepared using same protocols.

3.7 References

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